TRANSFUSION

Volume 1 September-October 1961 Number 5

Editorial

Science, Economics, Politics, and Blood

STRANGE bedfellows, these four. Your immediate reaction as a scientific or technical blood banker is that they should not appear together; they are sequentially antagonistic. I agree with the spirited nature of your reaction, but not with its factual practicality.

Science, economics, politics, and blood are related and there is logic in this sequence.

They have been arranged in this way for almost 25 years—ever since blood from a bottle was transfused into a vein. It was then that a blood transfusion was accepted as a practical and valuable medicinal agent because it became available in volume to many people. It was then that blood banks were born. However, it took blood banking 25 years of weed-like growth and about 60,000,000 units of blood to arrange these words for public view.

Unfortunately, the donor and the recipient are much more aware of this combination of words than the average blood banker. A few days ago a bill was introduced into Congress (HR 6416) which, if enacted, would allow the blood donor a $50 deduction (up to $200 per year) from his taxable income for each pint of blood which he gave, on the grounds that this act constituted a charitable contribution to society.

The cycle is now complete: *Science* was responsible for the discovery of the usefulness of blood in medical care; *Economics* is a necessary part of a transfusion; *Politics* became involved because blood "is for the common good"; *Blood* is the vehicle which welded these words together.

It does not matter for the purpose of this discussion whether the intent of the bill or its provisions are good or bad. What matters is that blood is added to the long list of health legislation sponsored by politicians who are constantly searching for the "common good" in order to bestow benevolence upon their constituents—for a price.

What is that price? All that the donor-politician expects to receive for his service is the "gratitude" of his constituents expressed in terms of votes. For the citizen-recipient of the "common good" the price is exorbitant—authoritarian control. A famous Supreme Court justice in a majority opinion on this subject which is currently the law of the land, said, "It is hardly less than due process that the government regulates that which it subsidizes." It can't be any other way. We blood bankers, as vendors of this blood service, could not escape being regulated.

The politician's wares must be attractive and of general interest in order to sell.

Today the most attractive item is the

health package: something which everyone wants; a civil right, a prominent cabinet member recently called it; something which the public-at-large has judged to be good. Good doctors, good hospitals, good polio vaccine, good liver extract, good antibiotics, good heart and brain surgery, good laboratories, good radiological services, and good blood, are all generally accepted as part of our way of life.

Blood is the last of these attractive items which the "politico" has placed in his show window. You thought it was in the blood banker's window! Actually it still is, but it won't be there for long unless blood bankers start doing more homework on the subjects of economics and politics.

If you don't believe this, look at the price tag he has on the bottle of blood. It is clearly marked FREE. You would get the shock of your life if you found the same tag on a suit or a hat in Macy's window. You would conclude that the management must be crazy. But, the politician isn't crazy. He and non-political groups who actively support the concept of blood for "gratitude" will get what they want unless we understand the economics of blood banking.

Not long ago, our Achilles' heel was the lack of essential information on many scientific aspects of blood banking: Rh, ABO subgroups, compatibility testing, etc. Right now, plain, hard economics – dollars – has added another Achilles' heel. We must continue our search for scientific truths but we must add to this, a search for socio-economic truths.

How much does it cost to give a transfusion? Is the public attracted by this FREE tag on the blood package? The answer is clearly "yes." They have already established this as a fact; not only from the standpoint of blood, but annually 35 million Americans walk into the Federal health store and receive a part or all of their health care. Many of the remainder are sorely tempted because they half-believe it is really free and half-believe that it ought to be.

Every sensible person knows that there is no such thing as a free service. Someone always pays because some part of that service is always rendered by persons whose livelihood depends upon it.

Simple explanations for this unrealistic viewpoint are that good health is a "birthright of every citizen," that "I ought to get something in return for my tax dollar," or that "everyone else receives a subsidy, why not me?"

Why doesn't the voter-citizen believe that food, shelter, clothing, and plumbing—all essential to good health—are also his vested right as a citizen? He understands the cost of these items because he is both a vendor and a consumer and belongs to the majority of people who are engaged in such activities. In sharp contrast to this group, the vendors of health benefits are a group of scientifically trained people who constitute only a small fraction of the consumer population. Their education is predominantly scientific both at the undergraduate and graduate level. People usually shun anything that they don't understand, and scientific people generally don't understand the business aspects of health finances.

What has all of this to do with a $50 deductible pint of blood? Simply this: Blood banking is now a part of the political and economic interest of a large number of American groups because they have adopted it as a common good which everyone should have. The only part of blood banking genuinely understood by them is the dollar part.

What can the members of the AABB do about it? We as blood bankers must understand them and they must understand us. Can this be done by the AABB governing board? Can this be solved by top-level conferences? NO, it cannot, because the top, like a tree, is only as strong as its roots.

Let us start with you. Can you survive a critical socio-economic examination of your individual bank? Can we as an organization pass this same examination? As an adolescent, the AABB (now 14 years old) would probably have difficulty with this examination because we have encouraged a diffusion of responsibility rather than central controls. We have developed guides, not dictates. Being able to experiment freely with various methods of rendering a blood service to people, we have gained strength and a position of leadership in this field. Our scientific and technical contributions to blood banking have been a hallmark of our success.

From a scientific standpoint, our journal TRANSFUSION, our Reference Laboratories, our scientific programs at the annual meeting, and the research contributions by our members have been outstanding.

From a technical standpoint, our annual technology panels, our Rare Blood Donor File, our nationwide workshops, our Inspection and Accreditation Program, our Technical Manual, and our "Standards for a Blood Transfusion Service" have brought a virtually "safe" transfusion to all who need one.

What about our third dimension, administration—another word for economics and politics? For years, your Board has spent 90 per cent of its time on administrative problems. About 60 per cent of your *Bulletin* contained printed matter directly related to this subject and yet can you logically substantiate the charge you make in your own facility for your blood service? Do you know whether the patients in the hospitals in your area are paying $20, $30, or even $60 for one transfusion? If you knew that there was a 100 per cent variation in the cost to patients, what would you do about it? Would you snipe at your sister bank and somehow naively believe (if your cost was higher) that you were all virtue and she is all bad because you thought your service was better?

My plea is that you first put your own house in order and find out whether your cost really reflects better professional and technical services and a better cost accounting practice, or is it merely because you have rationalized and not analyzed your charges?

Your Association leadership has given top priority to socio-economic matters for several years. Without AABB ownership of the clearinghouses, an AABB-ARC Intersociety Reciprocity Agreement (an economic achievement of vital importance) would have been impossible. There is no doubt in my mind that without this agreement, a unified, centrally controlled blood program would have soon evolved in which our policy-voice would have been a whisper. This agreement will lead to some confusion, some need for personal sacrifice of time and money until all of the problems come to light through its operation. These are a part of the sacrifices or justifiable "overhead" we must make because we adopted the principle that a diffusion of responsibility is an essential ingredient of freedom to act as our own master.

Our meetings with the Community Services Activities leaders of the AFL-CIO in New York City have resulted in an understanding at the "top." Now we have transferred this to the grassroots through the State Representatives and to blood banks directly concerned with the problem of the relationships of organized labor and blood banking at the community level. How well they are solved depends upon the knowledge of socio-economic affairs, the energy, and the skill of the person who directs the individual bank.

Our Committee on Blood Banking Systems has met twice and has succeeded in classifying donors. Their primary concern in this effort was not scientific or technical; it was politico-socio-economic.

Another committee charged with the responsibility of finding out what patients in this country pay for a transfusion, has al-

ready encountered great difficulties. Too many transfusion facilities have inadequate accounting practices and really don't know their costs.

Remember the politician I mentioned above? He didn't just offer the public a tax break by recommending that a blood donation be a deductible contribution. He did much more than that: he put a dollar value – $50 – on a pint of blood, for the blood itself.

Is this a scientific, technical, or a practical politico-economic problem? Have you determined the common denominators in your bank which will best serve your patients, your donors, and your bank? Remember that since World War II (Isn't it strange that this also marks the beginning of blood banking in this country?), scientists have made the discoveries and politicians have made the decisions as to what should be done with these discoveries?

Our future rests largely in the hands of our individual blood banks who must continue to be unified and fuse our scientific and technical know-how with a sound socio-economic approach to the solution of our problems.

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President

$\overline{\overline{R}}^{o}(D--)$ in Two Japanese Families with a Note on Its Genetic Interpretation*

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Members of two Japanese families residing in the same small village were shown to carry the $\overline{\overline{R}}^{o}$ (*D*- -) gene when antibodies in the sera of the two probands presented a crossmatching problem. A study of their sera revealed a complex array of Rh antibodies, including perhaps anti-*Hr*^{s}.

The $\overline{\overline{R}}^{o}$ (*D*- -) complex is considered to have arisen by mutation of several sub-elements (mutons) according to the cistron theory of genetopology.

THE FIRST example of the $\overline{\overline{R}}^{o}\overline{\overline{R}}^{o}$ (*D*- -/*D*- -) genotype was discovered by Race, Sanger and Selwyn[12, 13] in 1950. This unusual genotype was found in one family in the United States[18] and two families in Canada.[4-6, 10] More recently an additional case has been found in Japan.[11]

Characteristics of these bloods as established by previous studies are: (1) the absence of the **rh'** (C), **hr'** (c), **rh"** (E) and **hr"** (e) blood factors; (2) the heightened reactivity of the cells with anti-**Rh**$_{o}$ (D) sera as revealed by titration tests in parallel with "standard" **Rh**$_{o}$ (D) positive cells; (3) an increased capacity of the cells to absorb anti-**Rh**$_{o}$ (D) sera; and (4) the direct (saline) agglutination of $\overline{\overline{\mathbf{Rh}}}_{o}$ (D- -) cells by incomplete anti-**Rh**$_{o}$ (D) sera.

The present paper reports studies of two Japanese families of unmixed ancestry in which were seen persons homozygous and heterozygous for $\overline{\overline{R}}^{o}$ (*D*- -) and includes our efforts to identify the antibodies in the sera of some of them. A preliminary report of one of these families has been published elsewhere.[21]

Results

Investigation of the G. Family: The bloods of this family were brought to our attention by the discovery that the serum of the propositus, Mrs. G., agglutinated all bloods with which it was crossmatched. The medical history revealed previous abortions, stillbirths and multiple blood transfusions. When the serum of the propositus failed to agglutinate two known $\overline{\overline{R}}^{o}\overline{\overline{R}}^{o}$ (*D*- -/*D*- -) bloods,† it was suspected that her blood might also be of the same genotype.

Table 1 shows the results of tests done on cells from 17 members of the G. family (pedigree in Fig. 1) with two anti-**rh'** (C) and four anti-**rh"** (E) sera, all six of which were from **Rh**$_{o}$ (D) positive individuals, and with three anti-**hr'** (c) and two anti-**hr"** (e) sera.

The propositus, Mrs. G. (II,6), has five siblings and one living parent, as shown in Figure 1. Since two of these siblings (II,5 and II,7) were $R^{1}R^{2}$ (*DCe*/*DcE*), it was assumed that the deceased father (I,2) should have been $\overline{\overline{R}}^{o}\overline{\overline{R}}^{2}$ (*D*- -/*DcE*). Two other sibs (II,3 and II,4) also proved to be $\overline{\overline{R}}^{o}\overline{R}^{2}$ (*D*- -/*DcE*) and another (II,1) was $\overline{\overline{R}}^{o}\overline{R}^{o}$ (*D*- -/*D*- -). This latter sib, Mrs. N. (II,1) was further studied and her serum

* Received for publication March 20, 1961; accepted June 17, 1961.

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† Kindly supplied by Dr. Philip Levine, Ortho Research Foundation, Raritan, New Jersey

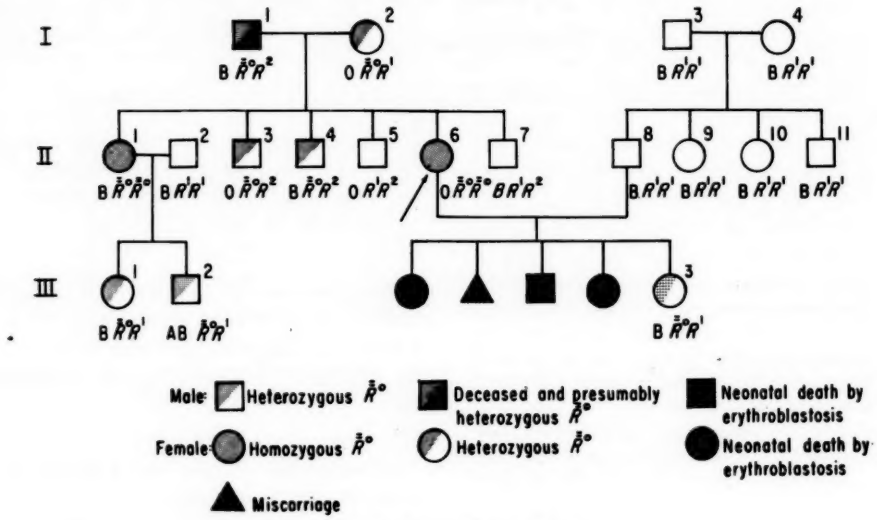

FIG. 1. Pedigree of the G. family.

was found to contain antibodies for all cells tested except those from the other known $\bar{R}^{o}\bar{R}^{o}$ (*D*--/*D*--) bloods, negative to the serum of Mrs. G., and those of Mrs. G. herself.

Some months later, the serum of Mrs. G. was again made available when she delivered her fourth living child (III,3). The baby manifested a positive direct antiglobulin test and a diagnosis of hemolytic disease of the newborn was established. The baby was successfully exchange-transfused with washed red blood cells from Mrs. N. (II,1), the sister of Mrs. G.

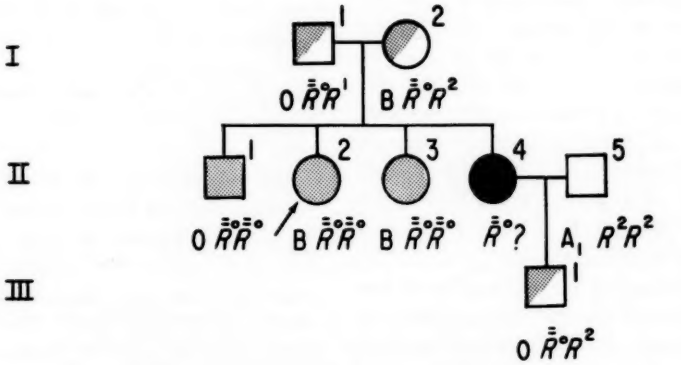

FIG. 2. Pedigree of the N. family.

TABLE 1. *ABO Blood Groups and Rh Genotypes of the G. Family*

Generation	A B O	Reaction with Anti-sera Rho(D)	rh′(C)	rh″(E)	hr′(c)	hr″(e)	Reaction*	genotype
I –1 †	(B)	Deceased						$(\bar{R}^{o}R^{2}(D\text{--}/DcE)$
–2	O	+	+	–	–	+	++	$\bar{R}^{o}R^{1}(D\text{--}/DCe)$
–3	B	+	+	–	–	+	–	$R^{1}R^{1}(DCe/DCe)$
–4	B	+	+	–	–	+	–	$R^{1}R^{1}(DCe/DCe)$
II –1	B	+	–	–	–	–	+++	$\bar{R}^{o}\bar{R}^{o}(D\text{--}/D\text{--})$
–2	B	+	+	–	–	+	–	$R^{1}R^{1}(DCe/DCe)$
–3	O	+	–	+	+	–	++	$\bar{R}^{o}R^{2}(D\text{--}/DcE)$
–4	B	+	–	+	+	–	++	$\bar{R}^{o}R^{2}(D\text{--}/DcE)$
–5	O	+	+	+	+	+	–	$R^{1}R^{2}(DCe/DcE)$
–6 ‡	O	+	–	–	–	–	+++	$\bar{R}^{o}\bar{R}^{o}(D\text{--}/D\text{--})$
–7	B	+	+	+	+	+	–	$R^{1}R^{2}(DCe/DcE)$
–8	B	+	+	–	–	+	–	$R^{1}R^{1}(DCe/DCe)$
–9	B	+	+	–	–	+	–	$R^{1}R^{1}(DCe/DCe)$
–10	B	+	+	–	–	+	–	$R^{1}R^{1}(DCe/DCe)$
–11	B	+	+	–	–	+	–	$R^{1}R^{1}(DCe/DCe)$
III –1	B	+	+	–	–		++	$\bar{R}^{o}R^{1}(D\text{--}/DCe)$
–2 §	AB	+	+	–	–		–	$\bar{R}^{o}R^{1}(D\text{--}/DCe)$
–3	B	+	+	–	–	+	++	$\bar{R}^{o}R^{1}(D\text{--}/DCe)$

* Agglutination in saline with incomplete anti-Rh_0(D) sera.

† Presumed ABO blood group and Rh genotype as he has died.

‡ Propositus.

§ Non-paternity.

Several attempts were made to identify the antibodies in the mother's serum. Inasmuch as the titer was relatively high (1:512 to 1:1,024) by the antiglobulin and trypsin technics, the results were unusual. After absorption with either Rh_1Rh_1 (DCCee), Rh_2Rh_2 (DccEE) or rh (dccee) cells, the remaining antibodies gave weak or doubtful reactions which were neither clear-cut nor meaningful. The presence of anti-**hr″** (E), at first thought to be definitely established, later became questionable in the light of elution data. Traces of anti-**hr′** (c) and anti-**hr″** (e) were also suggested.

Eluates of the cells exposed to the mother's serum gave unexpected reactions by Stern's papain test[17] and the indirect antiglobulin test. An antibody which directly agglutinated only the **rh″** (E) positive cells of our panel, including rh″rh″ (ddccEE), Rh_2Rh_2 (DccEE) and Rh_zRh_z (DCCEE), was found in eluates from rh (ddccee) cells, but not in eluates from Rh_2Rh_2 (DccEE) cells. These latter cells could, however, be shown to be strongly sensitized by the indirect antiglobulin method. The eluate from Rh_1Rh_1 (DCCee) cells agglutinated all cells tested except $\bar{R}^{o}\bar{R}^{o}$ (*D- -/D- -*). The cells of the propositus, Mrs. G., would not absorb antibody from her serum nor would they absorb antibody from several examples of anti-**rh′** (C), anti-**rh″** (E), anti-**hr′** (c) and anti-**hr″** (e).

The specificity of the antibodies in the baby's serum were also obscure and the baby's cells were not available for elution studies. The same surprising phenomenon of the elution of apparent anti-**rh″** (E) from

Generation	Reaction with anti-sera							Genotype
	Rho(D)	rh′(C)	rh″(E)	hr′(c)	hr″(e)	rh^w(C^w)	hr^s(V)	
I–1	+	+	–	–	+	–	–	R^oR^1(D--/DCe)
–2	+	–	+	+	–	–	–	R^oR^2(D--/DcE)
II–1	+	–	–	–	–	–	–	R^oR^o(D--/D--)
–2*	+	–	–	–	–	–	–	R^oR^o(D--/D--)
–3	+	–	–	–	–	–	–	R^oR^o(D--/D--)
–4	Deceased							
–5	+	–	+	+	–	–	–	R^2R^2(DcE/DcE)
III–1	+	–	+	+	–	–	–	R^oR^2(D--/DcE)

* Propositus.

TABLE 2. *Rh Blood Type of the N. Family*

rh″ (E) negative cells exposed to the test serum was also observed with the baby's serum.

Investigation of the N. Family: During the course of the work on the G. family, another blood representing a transfusion problem was submitted for study. Until the genotype of this blood was established, no compatible blood could be found for this patient. Another example of $\bar{\bar{R}}^o\bar{\bar{R}}^o$ (*D--/D--*) was quickly revealed when the serum failed to agglutinate the cells of Mrs. G., the propositus of the first family, and two additional $\bar{\bar{R}}^o\bar{\bar{R}}^o$ (*D--/D--*) bloods.

Absorption studies suggested the presence of multiple Rh antibodies. Eluates of the cells used for absorption gave anomalous results. As in the previous case, the eluate from rh (ddccee) cells agglutinated only rh″ (E) positive cells, the eluate from Rh_1Rh_1 (DCCee) cells agglutinated all cells tested except $\bar{\bar{R}}^o\bar{\bar{R}}^o$ (*D--/D--*), and the eluate from Rh_2Rh_2 (DccEE) cells contained no detectable antibody.

The pedigree of the N. family is given in Figure 2 and the blood typing results are tabulated in Tables 2 and 3. To the best of our knowledge the N. and G. families are not related. They do, however, reside in the same small village, and it is quite possible that at some earlier date, members of the two families were intermarried. We were unable to obtain information pertaining to consanguinity in either family, but consanguinity is common in Japan, especially in more rural communities.

Discussion

The question of the genetic origin of the $\bar{\bar{R}}^o\bar{\bar{R}}^o$ (*D--/D--*) genotype is yet an open one. Although the original communication of Race, Sanger and Selwyn[12] favored the operation of a chromosome deletion, the recent studies of Benzer[2, 3] with T4 bacteriophage have permitted a re-evaluation of the gene not only in terms of its various functions or effects but with regard to its qualitative structure as well. By employing recombination tests in tandem with "markers" obtained by observing spontaneous, and to a limited extent, chemically induced mutations, Benzer[2, 3] has been able to show that alleles, which he calls "cistrons," may themselves be subdivided. The cis-trans test serves to delimit the extremes of the cistron. The arrangement of the subelements, like that of the cistrons themselves, is linear and their topology and topography can be schematically mapped.

TABLE 3. *Blood Groups and Types of the N. Family*

Generation	A	A_1	B	M	N	S	s	U	He^a	P	K	k	Kp^a	Kp^b	Fy^a	Di^a	Le^a	Be^a	Jk^a
I–1	−	−	−	+	+	−	+	+	−	−	−	+	−	+	+	−	−	−	+
–2	−	−	+	+	+	−	+	+	−	+	−	+	−	+	+	−	−	−	+
II–1	−	−	−	+	+	−	+	+	−	−	−	+	−	+	+	−	−	−	+
–2*	−	−	+	−	+	−	+	+	−	+	−	+	−	+	+	−	−	−	+
–3	−	−	+	+	−	−	−	+	−	−	−	+	−	+	+	−	−	−	+
–5	+	−	−	−	+	−	+	+	−	−	−	+	−	+	+	−	−	−	+
III–1	−	−	−	−	+	−	+	+	−	−	−	+	−	+	+	−	−	−	+

* Propositus.

The smallest sub-element of a cistron that may be altered (*i.e.* mutates) is termed a "muton," and the cistron contains many of them. It has been further demonstrated that some mutons are extremely stable while others mutate easily.

Since DNA is now believed to be the carrier of the genetic code in most viruses and bacteria, the cistron-muton concept should be applicable to man and other higher forms by analogy, although most of the evidence linking DNA to hereditary transmission in higher forms is both incomplete and indirect. Applying this hypothesis, the Wiener and Fisher-Race theories of inheritance within the Rh system reduce to the same common denominator and become practically indistinguishable. The cistron, in conception, replaces both the single indivisible gene and the pseudoallelic elements of the two theories. An objection to this use of the word "cistron" in the place of "gene" has been offered by Carlson[7] on the basis of his work with the dumpy region in Drosophila. In this species, in contrast to T4 phage, compound mutants did not necessarily produce a mutant expression in the "trans" condition.

If one considers the Rh locus to be a single cistron divisible into mutable subunits, the explanation of $\overline{R}^o\overline{R}^o$ (*D- -/D- -*) as a relatively large mutation within the cistron becomes quite plausible. The biosynthetic pathways through which a mutation within the Rh cistron might exert its effect are unknown and, clearly, any efforts to elucidate them at this time are speculative. However, immunohematologists regard the production of the various Rh-Hr factors to be wholly the result of the action of their corresponding genetic determinant. In the ABO system, however, these factors may be the result of modification of a precursor substance by the cistron in question, an event that takes place at the terminal stage of their formation, at least for the secreted substances.

Regardless which, if either, of these two mechanisms operates, it may be assumed that the Rh cistron in some way controls the appearance of several Rh specificities. A mutation might affect one or more of these specificities depending on its magnitude, and in Benzer's terms, the number of mutons affected. At the moment there is no evidence assuring the existence of only one cistron, nor is there any indication that there may be more than one. Investigations designed to clarify this issue are hampered by the interaction of the Rh alleles of each chromosome, the difficulty in locating the original "marker" mutant and the lack, until recently, of sufficiently refined quantitative technics for measuring red cell antigen concentrations. We particularly stress in the latter instance, the work of Masouredis *et al.*[9] with I^{131} labeled Rh_o (D) antibody and the application of the probit bioassay technic of Wilkie and Becker[20] to the study of the Rh system by Silber *et al.*[16]

Race and Sanger[14] have recently noted an excess of consanguineous marriages in families possessing the $\overline{\overline{R}}^o$ (*D*- -) gene. They suggest that perhaps the $\overline{\overline{Rh}}_o$ (D- -) phenotype arises heterogeneously from the action of modifier genes, differing in each of these families. The ultimate proof of this hypothesis must await data furnished from pedigrees in which non-consanguineous matings of homozygotes and/or heterozygotes for $\overline{\overline{R}}^o$ (*D*- -) show whether the modifiers in these families, if they exist, differ from one another.

Anti-$\mathbf{Hr}_o$ (CcEe) is defined by Wiener *et al.*[19] as the antibody produced by sensitized persons homozygous for $\overline{\overline{R}}^o$ (*D*- -). Several investigations have now confirmed its existence, particularly that of Allen.[1] By the expedient of triply absorbing and eluting the antibody from Rh_1Rh_1 (DCCee), Rh_2Rh_2 (DccEE) and *rh* (ddccee) cells in succession, they showed that the final eluate contained antibodies having a new specificity and directed toward a nearly universal factor, $\mathbf{Hr}_o$ (CcEe), present on all but $\overline{\overline{R}}^o\overline{\overline{R}}^o$ (*D*- -/*D*- -) cells. Thus, an injection of red blood cells into a person of $\overline{\overline{Rh}}_o$ (D- -) phenotype might elicit anti-$\mathbf{Hr}_o$. It might also be argued that anti-$\mathbf{Hr}_o$ (CcEe) represents an antibody of broad cross-reactivity rather than being directed against a single factor. There seems to be little reason yet to favor one hypothesis over the other in the absence of controlled injection experiments. Hackel[8] has described antibodies with unusual specificity that are produced by $\overline{\overline{R}}^o\overline{\overline{R}}^o$ (*D*- -/*D*- -) persons.

The results of our studies do not indicate that anti-$\mathbf{Hr}_o$ (CcEe) might have been absent from the sera reported in this paper, since "triple eluates" as described by Allen[1] were not attempted.

The antibodies eluted from rh (ddccee) cells agglutinated all **rh″** (E) positive cells including Rh_zRh_z (DCCEE). No antibody could be eluted, however, from Rh_2Rh_2 (DccEE) cells although these cells could be shown to have absorbed antibody by the indirect antiglobulin test. Thus, although the agglutination specificity of an eluate resembles anti-**rh″** (E), this conclusion may be precluded by the absorption and elution of the antibody with **rh″** (E) negative cells. Such a result could be tentatively attributed to a specificity such as $\mathbf{Hr}^s$,[15] with the antibodies having a stronger affinity for **rh″** (E) positive cells than for **rh″** (E) negative cells.

Acknowledgments

The authors would like to express their appreciation to Dr. Philip Levine for advice in the interpretation of these data.

Note

A reinvestigation of the N. family has revealed an error in the pedigree (Figure 2). Mrs. S. (II, 4) is not deceased; her blood type is O $\overline{\overline{R}}^o\overline{\overline{R}}^o$ (*D*- -/*D*- -). Three additional sibs of Mrs. N. (II, 2) were also found and two were tested. These proved to be *O* R^1R^2 (*DCe/DcE*) and *B* $R^1\overline{\overline{R}}^o$ (*DCe/D*- -) respectively.

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Citrate-Phosphate-Dextrose Solution for Preservation of Human Blood:*

A Further Report

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A comparison of clinically significant *in vitro* characteristics of ACD and CPD blood is presented.

In clinical trials conducted at three hospitals, 586 units of blood collected in CPD in plastic bags were utilized for 622 individual treatments as single units or subdivided aliquots of whole blood, and as packed red cells in 332 patients. The expiration period was 28 days, and five bloods were discarded as outdated. There was one reported pyrexial reaction and one proven bacterial contamination.

Forty-two per cent of the bloods or components were issued during the first week, 43 per cent during the second and third weeks, and 15 per cent during the fourth week of storage.

Of the total bloods or components issued, 9 per cent of the whole bloods, 25 per cent of the subdivided blood aliquots, and 23 per cent of the packed red cells were transfused during the fourth week of storage.

No difficulty with clotting was observed during collection, storage, or transfusion.

A Citrate-Phosphate-Dextrose (CPD) anticoagulant solution for the preservation of human blood was first described in 1957.[7] This solution differs from Acid-Citrate-Dextrose (ACD) solution, in that it contains 20 per cent less citrate ion; contains dibasic sodium phosphate; is isotonic to human red cells, and has a *p*H of 5.63.

The formula of the CPD† solution used in all experiments and clinical trials reported herein is as follows:

	Gm.
$Na_3\,C_6\,H_5\,O_7{:}2H_2O$	25.80
$H_3\,C_6\,H_5O_7{:}H_2O$	3.20
$Na\,H_2\,PO_4{:}H_2O$	2.18
$C_6\,H_{12}\,O_6$	25.00
Water for injection	1,000.00
*p*H	5.63
70 Ml. for collection of 500 Ml. of blood	

All of the bloods used for the *in vitro* and *in vivo* experiments, and the clinical trials reported herein, were collected and stored in plastic bags;†† and transfusion was by plastic recipient sets.

In the original publication,[7] data were presented on the post-infusion survival, determined by the radiochromium (Cr^{51}) method,[6]††† of red cells of whole blood collected in CPD and stored at 4 ± 1 C. for from 27 to 32 days. In eight experiments the percentage survival ranged from 70 to 85 per cent with a mean of 75 per cent. Since these were reported, three survival experiments were carried out on bloods collected in plastic bags which had been stored at room temperature in the original package for three years, with results in the same range.

* Received for publication January 16, 1961; accepted April 12, 1961.

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This work was supported by a grant-in-aid from the National Heart Institute of the National Institutes of Health, and from Fenwal Laboratories, Inc., Framingham, Massachusetts.

† This is the formula of CPD-5 in the original article.[7]

The results of these 11 experiments with CPD blood, shown in comparison with seven survival experiments on ACD bloods, processed with similar equipment,[10] are shown in Figure 1. At 28 days of storage, the range for the ACD bloods was from 63 to 75 per cent with a mean of 70 per cent, three values being well below this level. It is evident that ACD blood, even when collected in plastic equipment, does not, after 28 days of storage, meet with the National Institutes of Health[4] requirement of 70 per cent survival. In contrast, ten of 11 CPD bloods had values of 70 per cent or better.

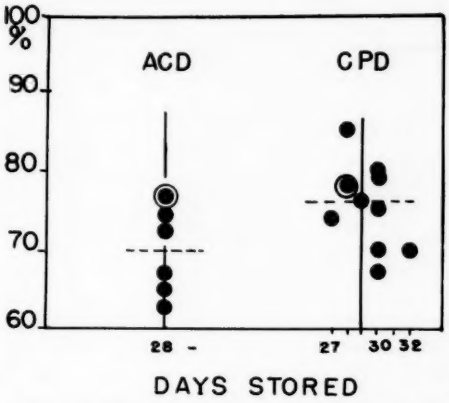

FIG. 1. Comparison of post-transfusion survival of red cells of ACD and CPD blood collected and stored in plastic bags. The mean survival at 28 days is 70 for ACD and 76 per cent for CPD cells. Three ACD bloods had values below, while 10 CPD bloods had values above the N.I.H. limit of 70 per cent.

Since ACD is the currently accepted anticoagulant solution for the collection of blood for bank storage, a comparison of *in vitro* characteristics of ACD and CPD blood (not hitherto reported) that are of clinical significance is presented.

In Table 1 are shown comparative values of plasma *p*H, hemoglobin, potassium, and inorganic phosphate concentration over a storage period (4 ± 1 C.) of 35 days. These values were obtained on paired aliquots of blood from the same donor collected in the two solutions.

Plasma *p*H was determined with a glass electrode using a Beckman Model G *p*H meter. Hemoglobin was measured by the method of Crosby;[3] potassium with a Baird flame photometer, and inorganic phosphate by the method of Fiske and SubbaRow.[5]

The initial *p*H of ACD plasma is 7.0 and falls rapidly to 6.8 at seven days, and thereafter less rapidly to 6.7 at 21 days. The initial *p*H of CPD plasma is 7.2; and although there is a progressive fall, the level at 35 days of storage is higher than that of ACD blood at 21 days. CPD blood contains less fixed acid throughout the entire period of storage.

The plasma hemoglobin concentrations are less in CPD than in ACD plasma throughout storage, the CPD level at 28-days storage being considerably lower than that of ACD blood at 21 days of storage.

The net loss of intracellular potassium and net gain in plasma potassium is significantly less in CPD than in ACD blood, and plasma potassium levels are lower throughout storage.

The formulaic inorganic phosphate of CPD results in a higher initial plasma level than in ACD blood. In both bloods the breakdown of intracellular organic phosphates during storage results in a diffusion of extracellular inorganic phosphate, and as a result, the plasma inorganic phosphate level of CPD blood exceeds that of ACD throughout storage, as shown in Figure 2. In ACD cells the level rises rapidly up to 15 days, indicating a rapid breakdown of organic phosphate; thereafter the level re-

†† Supplied by or purchased from Fenwal Laboratories, Inc.; JA2C containing ACD, and JF5 containing CPD.

††† In this method the 100 per cent retention value of infused stored red cells is based on the recipient's red cell volume determined with fresh recipient's red cells tagged with P^{32}, and the measured amount of Cr^{51} administered as tagged donor cells. Post-infusion survival is based on a value obtained by extrapolation of survival percentages of individual samples taken over a 20-day period plotted as semi-logarithmic values.

TABLE 1. *Comparison of plasma pH, and Plasma Concentrations of Hemoglobin, Potassium and Inorganic Phosphate of ACD and CPD Blood*

Days stored	0	7	14	21	28	35
			*p*H			
ACD	7.00	6.79	6.73	6.71	6.71	6.67
CPD	7.20	7.00	6.89	6.84	6.78	6.74
			Hemoglobin Mg/100 Ml.			
ACD	10	22	35	53	81	150
CPD	4	10	21	30	40	54
			Potassium MEq./L			
ACD	10	20	29	35	40	42
CPD	3	10	20	25	28	30
			Inorganic Phosphate mM/L			
ACD	0.6	1.5	2.2	3.0	3.4	3.8
CPD	3.6	3.6	4.2	4.9	5.5	5.8

mains unchanged, indicating a failure of glycolytic activity. In contrast, the intracellular level of CPD cells rises slowly during the first 15 days, indicating conservation of organic phosphate. Thereafter, the level continues to rise up to 28 days, indicating continued glycolytic activity.

This metabolic difference has been further investigated by phosphate partition studies on paired aliquots of whole ACD and CPD blood from the same donor.[11] Half of the anticoagulant solution from each of two ACD and CPD collection bags was removed and discarded. Two donors were bled: half of the unit from each donor being collected in CPD solution, and the other half in ACD.

Phosphate partition hydrolysis studies were carried out by the method outlined by LePage.[8] This procedure involves a fractional analysis of the organic phosphate

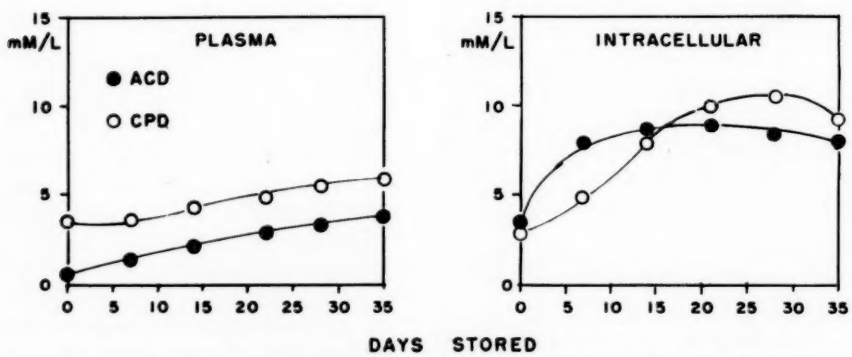

FIG. 2. The level of plasma inorganic phosphate of CPD blood exceeds that of ACD blood by about 2 mM/L throughout storage. Intracellular phosphate of ACD blood rises rapidly to a maximum in the first two weeks of storage, whereas that of CPD blood rises more slowly but progressively for 30 days. (We are indebted to Dr. P. R. Beamer, Editor of the American Journal of Clinical Pathology, and to Williams and Wilkins Co., Baltimore, Maryland, for permission to reproduce this Figure from a previous communication.[7])

esters present in a trichloracetic acid filtrate.

Samples of the deproteinized blood were subjected to hydrolysis with 1N HCl at 100 C. Inorganic phosphate before and after treatment was determined by the method of Fiske and SubbaRow.[5] The increase in inorganic phosphate during the first seven minutes of hydrolysis (P_L) is mainly due to the two labile phosphates of ATP and the labile phosphate of adenosine diphosphate. The additional amount liberated at the end of 100 minutes (P_H) is derived primarily from hexose diphosphates and adenosine monophosphate. Total acid soluble phosphate was determined by wet ashing with perchloric acid. The difference between the total hydrolyzable phosphate and the total acid soluble phosphate (P_{Total}) represents the stable phosphate fraction (P_S) which is essentially 2,3-diphosphoglycerate (DPG).

The results are given in Table 2. The breakdown of organic to inorganic phosphate in CPD blood proceeds at a significantly slower rate than in ACD blood, as shown in Figure 3, in which the values plotted represent net changes. Further study of the data indicate that this difference is due to a slower rate of loss of hydrolyzable phosphate, and, that since the rate of loss of labile phosphate is about

TABLE 2. *Phosphate Partition Studies on Paired Aliquots of ACD and CPD Bloods*

Unit No.		Days stored				
	ACD	*0*	*7*	*14*	*21*	*28*
N1724-0	P_I	0.94	3.00	4.83	5.13	5.36
	P_L	1.36	1.29	1.07	1.30	1.31
Phosphate	P_H	0.99	1.13	0.61	0.46	0.22
(mM/L)	P_S	4.69	2.03	1.35	0.90	0.99
	P_{TO}	7.04	4.45	3.03	2.66	2.42
	P_{Total}	7.98	7.45	7.86	7.79	7.78
	CPD					
	P_I	2.86	3.03	4.31	5.61	6.19
	P_L	1.50	1.45	1.05	1.06	1.37
	P_H	1.08	1.31	0.71	0.35	0.82
	P_S	4.55	3.98	4.11	2.58	1.74
	P_{TO}	7.13	6.74	5.81	4.07	3.93
	P_{Total}	9.99	9.77	10.18	9.68	10.12
	ACD					
N1723-0	P_I	1.07	3.05	4.94	4.95	5.98
	P_L	1.21	1.28	1.20	1.56	0.92
Phosphate	P_H	0.95	0.93	0.41	0.73	0.55
(mM/L)	P_S	5.25	3.18	1.90	1.47	0.88
	P_{TO}	7.41	5.39	3.51	3.66	2.45
	P_{Total}	8.48	8.44	8.45	8.61	8.43
	CPD					
	P_I	2.81	2.90	3.65	5.22	6.80
	P_L	1.32	1.15	0.93	0.93	1.04
	P_H	1.01	1.31	0.78	0.81	0.54
	P_S	5.38	5.09	5.16	3.56	2.14
	P_{TO}	7.71	7.53	6.87	5.30	3.72
	P_{Total}	10.52	10.43	10.52	10.52	10.52

P_I = Inorganic Phosphorus
P_L = Labile Phosphorus
P_H = Hydrolyzable Phosphorus
P_S = Stable Phosphorus
P_{TO} = Total Organic Phosphorus
P_{Total} = Total Acid Soluble Phosphorus

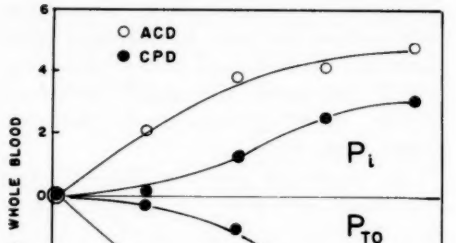

FIG. 3. The net loss in total organic phosphate and increase in inorganic phosphate is significantly less in CPD than in ACD blood throughout storage. The difference is due to a slower rate of loss of stable phosphate (DPG).

equal in both bloods, to a slower rate of loss of stable phosphorus (DPG).

This conservation of the phosphate ester pool appears to correlate with a decreased rate of loss of viability during storage.

Clinical Trials

Clinical trials of CPD have been conducted at the Maine Medical Center, Portland, Maine, the Children's Hospital Medical Center, Boston, Massachusetts, and the Harrisburg Hospital, Harrisburg, Pennsylvania. A dating period of 28 days was used in all three institutions. Whole blood only was transfused at the Maine Medical Center, and whole blood and packed red cells at the Harrisburg Hospital. Blood is collected in double bags* at Children's Hospital Medical Center, permitting aseptic component separation, and data are available on transfusion of whole units, and units subdivided into 250 and 125 ml. aliquots of blood, packed cells, and plasma.

A total of 90 CPD bloods were collected at Maine Medical Center during the period of April 18 to April 26, 1960. No difficulty in collection was encountered, and no clots were observed on storage. Eighty-three bloods were examined for plasma hemoglobin at the time of transfusion. Of these, seven had measurable amounts (0.09 to 0.39 Gm. %) and all others were reported negative.

A total of 50 patients were transfused. Bloods were issued for routine use and no selectivity as to type of case was attempted, the diagnostic categories being as follows:

Surgery: general	15	
open heart	2	
malignancy	18	
Cesarean Section	3	38
Fracture		1
G. I. bleeding		5
Post-partum bleeding		2
Uremia		3
Erythroblastosis (exchange)		1
Total		50

No reactions attributable to the administration of CPD blood were reported. It was the expressed opinion of the anesthesiologist, surgeons, and internists that the bloods were well tolerated.[9]

At the Children's Hospital Medical Center 500 CPD bloods were collected between June 27 and August 18, 1960, the disposition thereof being as follows:

Transfused as 500 ml. units		296
Subdivided to yield for individual treatments		188
Whole blood—		
250 ml. aliquots	121	
125 ml. aliquots	20	
Packed red cells	81	
Total treatments	222	
Not used		16
Research	7	
Outdated	5	
Mechanical difficulty	1	
Clotting	1	
Incomplete bleeding	1	
Contamination	1	
Total		500

* Fenwal Double Pack JD-2.

These 484 utilized bloods provided 518 treatments for 272 patients, of whom 64 were infants, 199 children (up to 18 years), and nine adults. Eighty-two units of plasma were obtained as a by-product of preparation of packed red cells.

No difficulty was experienced with formation of clots in stored blood. The supernatant plasma of fully sedimented CPD blood at 28 days showed no hemoglobin on visual inspection. One pyrexial reaction was reported.

The CPD blood was well tolerated as evidenced by the absence of disturbance in hemodynamics or impaired renal function, even in cases in which the ratio of blood transfused to estimated total blood volume was high.

At the Harrisburg Hospital 12 CPD bloods were administered to ten patients. Four given as whole blood had been stored 17, 21, 23, and 25 days, and eight given as separated cells had been stored 16 (2), 17, 20, 21, 24, and 27 (2) days.[1]

There were no hemolytic or pyrexial reactions. Plasma hemoglobin on the day of transfusion ranged from 1 to 10 mg. per 100 ml. with no correlation of level with storage period.

The increase in hematocrit ranged from 1 to 4.5 volumes per cent, averaging 3.6, a figure representing a high percentage of transfused cells remaining in circulation. This finding was substantiated by the very slight increases in serum bilirubin measured post transfusion.

In these three institutions, 586 CPD bloods provided 622 individual treatments as single units or aliquots of subdivided whole blood, and packed red cells in 332 patients.

In Table 3 the distribution of transfusions, given as whole blood, subdivded aliquots, or packed cells, is shown in relation to the storage period at the time of administration. The largest percentage of bloods (42.1) were issued during the first week of storage; an additional 43.3 per cent during the second and third week, but 14.8 per cent were used during the fourth week. Further analysis of the 91 issued during the fourth week, reveals that single units of whole blood (500 ml.) represents 9 per cent, subdivided aliquots of whole blood (250 ml. and 125 ml.) 25 per cent, and packed cells 23 per cent of the total in each category.

Many of the patients in these series received multiple transfusions. In the Maine Medical Center series, 22 patients received from one to six transfusions. The period during which individual patients were transfused varied from one to eight days. One patient received 6, one 5, and one 3 units of blood within 24 hours. Bloods given as multiple transfusions to an indi-

TABLE 3. *Distribution of CPD Bloods Transfused as Single Units or Subdivided Aliquots of Whole Blood, and as Packed Red Cells in Relation to Storage Period*

	Maine Medical Center	Children's Hospital Medical Center				Harrisburg Hospital			
Days stored		Whole blood			Packed cells	Whole blood	Packed cells	Total	Per cent
	500 ml.	500 ml.	250 ml.	125 ml.					
0-7	29	178	42	4	8	—	—	261	42.1
8-14	13	62	19	7	27	—	—	128	20.7
15-21	32	37	32	4	29	3	5	142	22.6
22-28	16	19	30	5	17	1	3	91	14.8
	90	296	123	20	81	4	8	622	100.2

TABLE 4. *Multiple Infusions of CPD Blood in Open-Heart Surgery*
(From the service of Dr. Dwight E. Harken, Peter Bent Brigham Hospital, Boston, Massachusetts)

Hospital number	Sex	Diagnosis	Operation	Units given	Days stored	Time Hours	Time Days
9P95	M	Aortic stenosis	Valve replacement	5	4	2	
7N695	M	Atrial myxoma	Cardiotomy	6	2	2	
7N768	M	Aortic stenosis	Valvuloplasty	9	1	4	
4P915	M	Mitral stenosis	Valvulotomy	9	3	3	
9P108	F	Mitral stenosis	Valvulotomy	17	15–1 2–2	4	
7P298	F	Mitral stenosis	Valvulotomy	18	9–1 2–2 7–3		2
9P193	M	Mitral insufficiency	Valvulotomy	18	4–1 14–3		1
4P786	F	Aortic stenosis	Valvulotomy	18	4–1 14–4	6	
4P808	M	Aortic insufficiency	Valve replacement	25	5–1 20–4		2
5L692	F	Multi-valvular stenosis	Valvulotomy	26	20–1 6–4		2
			Total	151			

vidual were selected so as to be as nearly of the same storage period as possible. Five patients requiring more than two units received bloods stored six days or less, but five patients in the same category received bloods stored from 13 to 20 days. It is of interest to note that one patient received five units of 20-day-old, and one 6 units of 15-day-old blood. These transfusions were equivalent to about half and two-thirds of the estimated normal total blood volume.

An even more striking situation was apparent in the Children's Hospital Medical Center series. In 261 patients the ratio of total infused whole blood, packed cells, or plasma to normal blood volume was calculated, the values of Brines,[2] *et al.* being used. Seventy-three per cent of the group received amounts ranging up to normal total blood volume, 18 per cent up to double, 6 per cent up to triple, 2 per cent up to quadruple, and 1 per cent up to quintuple replacement of total blood volume.

Had a 21-day dating period been applied, it is probable that many of these bloods or separated components would have been wasted. Since CPD blood meets the National Institutes of Health[4] requirements of 70 per cent post-transfusion survival after storage for 28 days, its use should greatly reduce loss of blood through outdating.

Open Heart Surgery

CPD blood has been successfully used for maintenance of blood volume during open heart surgery on the service of Dr. Dwight E. Harken, Peter Bent Brigham Hospital. The pump-oxygenator was primed with freshly drawn heparinized blood.

The quantity and number of days stored, administered in ten cases, is shown in Table 4. A total of 151 bloods were administered. While most of these were collected on the day of operation, the use of CPD blood stored under routine blood bank conditions up to four days was apparently effective and was not attended with untoward hemorrhagic effect.

Acknowledgments

The authors wish to express their appreciation for permission to present data obtained during clinical trials to Dr. Joseph E. Porter, Pathologist at Maine Medical Center, Portland, Maine; Dr. Alan Richardson Jones, Director of the Blood Transfusion Service, Children's Hospital Medical Center, Boston, Massachusetts; Dr. Herbert Bowman of Harrisburg Hospital, Harrisburg, Pennsylvania; and to Lt. (jg) John W. Zemp, Blood Research Laboratory, U. S. Naval Hospital, Chelsea, Massachusetts, for permission to present data on his phosphate partition studies of ACD and CPD blood.

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Thymol Turbidity and Thymol Flocculation Tests As a Screening Technic to Detect Blood Donors Carrying the Hepatitis Virus:*

I. The Results of Screening 52,662 Consecutive Blood Donors

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The thymol turbidity test was done by the Shank-Hoagland (*p*H 7.55) method and flocculation tests were done on all lipemic serum over 5.0 T.T. units. The mean for the readings on clear (nonlipemic) sera (87%) was 3.8 units, and the standard deviation 2.1. The screening level of abnormality at the 95% confidence level was 8.0 T.T. units in clear sera (or 8.0 T.T. units with positive flocculation in lipema sera), and 10.1 T.T. units (also with T.F. in lipemic sera) at the 99% confidence level. There were 5.4% and 1.7% "abnormal" bloods, respectively. The values for all sera, including lipemic, were: mean 4.4, S.D. 2.7, or 9.9 T.T. units at the 95% confidence level and 12.5 at 99%. A parallel study of the use of inactivated sera for the T.T. test is discussed. It lowered the T.T. values an average of 3.7 units but produced a more abnormal distribution of test results. Since the donors were healthy adults representing a good cross section of the adult population and the levels of abnormality (95 and 99% confidence) found for the test were considerably higher than that reported by most authors, the validity of the use of lower levels to screen blood donors is questioned. The use of large numbers of acceptable blood donors may be a better way to establish normal and abnormal values for clinical laboratory tests. The incidence of hepatitis in transfused patients, followed for six months, is reported in Part II.

A NUMBER of articles have appeared during the past several years relating to the use of the thymol turbidity test to screen blood donors in an effort to detect significant numbers of those who may be carriers of the serum hepatitis virus. In general, each author has employed a different screening level, but always higher than the "normal" value of five units (fasting subjects) and sometimes lower than eight units (nonfasting subjects).[8] However, with two exceptions, the data on which such selection was based have not been made available for evaluation, and in only one report has the level chosen been based on studies of the test in blood donors. Neefe and associates[7] consider 5.5-6.6 units (mean + 2 to 3 S.D.) as equivocal and over 6.6 (mean + 3 S.D.) as definitely abnormal, based on an earlier study of normal, nonfasting, young adults by the Shank-Hoagland method (*p*H 7.55). Jennings and associates[2] employed a screening level of eight Shank-Hoagland units or higher as abnormal, and Strumia and associates[11] employed 6.8 units, but neither gave any supporting data. Maxwell[4] used a value of 9.3 T.T. units (Shank-Hoagland), based on a 96.3 per cent confidence level in tests on slightly more than 10,000 blood donors.

The observations to be reported are based on the results of the thymol turbidity test values determined in 52,662 consecutive blood donors, during the two-year period of the study, who were acceptable at the time they presented themselves according to nationally approved standards.[9, 10] This represents approximately 21,300 individuals, based on a review of the number of previous donations given in the preceding 12-month period by every tenth donor during the year 1959. This indicates there were approximately 8,000 who gave only once

* Received for publication March 4, 1961; accepted June 3, 1961.

during the two-year period, the remaining 13,300 gave an average of 3.35 times, giving a total average of 2.47 donations per donor. It is appreciated that this is a study of serial blood donations rather than a series of individual donors. On the other hand, it does reflect accurately the thymol values of the total blood units available for use, and the number of individuals involved is large enough to prevent any appreciable weighting of the total picture by repeat ("abnormal") donors.

Materials and Methods

All tests were carried out with the Bausch and Lomb photocolorimeter, Spectronic 20, using Harleco (dry) Thymol Buffer #29951 (*p*H 7.55), which was prepared, stored and used according to the instructions furnished by the manufacturer, modified to conform with Reinhold's[8] excellent discussion of the thymol test technic. The only variation was that we did not use a 25 C. water bath for the test tube cuvettes, since the laboratory temperature was quite constant (forced air-air conditioning). The photocolorimeter was standardized at zero daily, using the thymol reagent as the unknown. The donor's serum was separated from the cells within 24 hours, usually within one to four hours, stored at 4-6 C. but allowed to reach room temperature for testing. The thymol turbidity (T.T.) reading was recorded in Shank-Hoagland units (*p*H 7.55) for all bloods, using normal (uninactivated) serum.** In recording the test results, a

** For reasons to be discussed later, during the second of the two years of testing all sera with a T.T. value over five units were retested, using inactivated sera.

TABLE 1. *Distribution of T.T. Values in 52,662 Donors*

T.T. reading	All tests	% of total bloods	Total nonlipemic	% of total nonlipemic	Per cent of nonlipemic in total bloods
0.0 – 0.9	1,383				
1.0 – 1.9	5,531				
2.0 – 2.9	10,468	68.7	36,139*	78.7	68.6
3.0 – 3.9	11,671				
4.0 – 4.9	7,086				
5.0 – 5.9	5,483		3,978		
6.0 – 6.9	3,703	21.8	2,762	18.1	15.8
7.0 – 7.9	2,309		1,583		
8.0 – 8.9	1,712		551		
9.0 – 9.9	1,178		362		
10.0 – 10.9	592		175		
11.0 – 11.9	461		123		
12.0 – 12.9	332		94		
13.0 – 13.9	210		38		
14.0 – 14.9	124	9.5	34	3.2	2.8
15.0 – 15.9	95		17		
16.0 – 16.9	116		18		
17.0 – 17.9	53		16		
18.0 – 18.9	36		12		
19.0 – 19.9	15		5		
Over – 20.0	104		15		
Totals	52,662	100.0	45,922	100.0	87.2**

* Lipemia not recorded below 5.0 T.T. units.
** Lipemic sera comprised 12.8% of all bloods.

TABLE 2. *Thymol Flocculation on Lipemic Bloods over 5 T.T. Units*

T.T. reading	Number lipemic	% of total lipemic bloods	% of total bloods	Number positive T.F.	% of lipemic bloods with positive T.F.	% of total bloods
5.0 – 5.9	1,505			257		
6.0 – 6.9	941	47.1	6.0*	230	10.0	1.3
7.0 – 7.9	726			186	(21.2 of group)	
8.0 – 8.9	1,161			354		
9.0 – 9.9	816			302		
10.0 – 10.9	417			164		
11.0 – 11.9	338			144		
12.0 – 12.9	238			114		
13.0 – 13.9	172	52.9	6.8**	70		
14.0 – 14.9	90			46		
15.0 – 15.9	78			37	20.2	2.6
16.0 – 16.9	98			48	(33.6 of group)	
17.0 – 17.9	37			17		
18.0 – 18.9	24			14		
19.0 – 19.9	10			5		
Over – 20.0	89			49		
Totals	6,740	100.0	12.8	2,037	30.2	3.9

* These comprise 27.6% of all bloods in the 5.0 to 7.9 T.T. group.
** These comprise 73.7% of all bloods in the 8.0 T.T. and over group.

notation "L" was made if the serum was lipemic (turbid) and the T.T. value was five units or more. The presence of lipemia was a visual judgment as to whether the serum was clear or showed distinct turbidity. The thymol flocculation test (T.F.) was done for confirmation on lipemic sera reading more than 5 T.T. units (the fasting normal) by allowing these tests (tubes) to stand for 24 hours at room temperature for the T.F. reading. It is well known[6, 8] that lipemic sera will give false positive T.T. tests and that the T.F. test is quite useful in determining which are true positives.

Results

Distribution of Thymol Turbidity Values: A considerably wider variation in T.T. values was observed in this series of 52,662 acceptable, apparently healthy, blood donors than was anticipated, as seen in Table 1. The results are shown separately for all tests (including lipemic sera) and for the 45,922 nonlipemic sera (87.2% of the total). Since the turbidity test is not reliable with lipemic sera, the 6,740 sera with T.T. values of five and over (12.8%) were segregated and tested by the flocculation method. The distribution of the turbidity test values in clear sera shows that 78.7 per cent of donor bloods were below 5 T.T. units, 18.1 per cent between 5 and 7.9 T.T. units and 3.2 per cent were 8 or more.

Thymol Flocculation Results in Lipemic Sera with Over 5 T.T. Units: Table 2 shows the results of the thymol flocculation test on the 6,740 lipemic sera. In the 5 to 7.9 T.T. group, 21.2 per cent were positive, and in those over 8 T.T. units, 33.6 per cent were positive. However, the 673 positive flocculation tests between 5 and 7.9 T.T. units are but 1.3 per cent, and the 1,364 positive tests in those with 8 T.T. units or more are 2.6 per cent, of the total number of bloods examined.

TABLE 3. *Table of Significant T.T. Test Values*

	Mean	Standard deviation	+ 2 S.D. 95% confidence level	+ 3 S.D. 99% confidence level
All tests	4.4	2.7	9.9 T.T. units and over 95.9% of tests	12.5 T.T. units and over 97.1% of tests
Nonlipemic tests	3.8	2.1	8.0 T.T. units and over 96.7% of tests*	10.1 T.T. units and over 99.4% of tests**

* As shown in Table 4, this becomes 94.6% of tests, when the results of the flocculation tests in lipemic sera are included.

** This becomes 98.3% of tests, when the results of the flocculation tests in lipemic sera are included.

Means and Standard Deviations: The mean and standard deviation (S.D.) have been calculated for purposes of comparison both for the total group of 52,662 test sera and for the 44,259 nonlipemic test sera. These values and the levels of significant abnormality are shown in Table 3. Although the values shown for the total group of bloods tested (mean = 4.4, S.D. = 2.7) are artificially elevated for the T.T. test because of the inclusion of all the lipemic sera, one can observe, comparing the two, that the distribution of lipemic sera was relatively proportional and did not greatly change the per cent of tests embraced within the + 2 S.D. and + 3 S.D. limits; also, the mean of all bloods available is useful for evaluation of the distribution of the bloods received by patients who were selected for the follow up study. The values (mean = 3.8, S.D. = 2.1) shown for the nonlipemic sera were used to establish the significant screening level. They indicate that the frequency distribution of the tests lies within reasonable limits of expectation. Because both levels have been employed for screening donors,[4,7] we used both + 2 S.D. (the 95% confidence level), the value at which the test results are probably significantly abnormal, and + 3 S.D. (the 99% confidence level), the value at which results are definitely abnormal. Therefore, T.T. values of 8.0 units or more in clear sera or 8.0 units or more with a positive flocculation test in lipemic sera (a combined total of 94.6% of all tests), and 10.1 T.T. units

TABLE 4. *Distribution of 52,662 "Normal" and "Abnormal" Bloods at Screening Level of 95% Significance**

T.T. group	"Normal"			"Abnormal"		Per cent	
	Clear	Lipemic without flocculation	Lipemic with flocculation	Clear	Lipemic with flocculation	"Normal"	"Abnormal"
0.0 to 4.9	36,139	Not recorded	Not recorded	0	Not done	68.6	0
5.0 to 7.9	8,323	2,499	673	0	0	21.8	0
8.0 and Over	0	2,204	0	1,460	1,364	4.2	5.4
Totals		49,838			2,824	94.6	5.4**

* Clear sera 8.0 T.T. units or more; lipemic sera 8.0 T.T. units or more, with positive T.F. test.

** At the 99% level of significance, there were 895 abnormal bloods (1.7%). The great majority of these were lipemic with a positive T.F. test.

TABLE 5. *Effect of Inactivation of Serum on the T.T. Test**

T.T. reading	Unheated	Heated**	% unheated	% heated
0 – 4.9	—	4,864	0	54.9
5.0 – 5.9	2,936	1,598		
6.0 – 6.9	1,983	813	69.6	33.4
7.0 – 7.9	1,236	548		
8.0 – 8.9	917	392		
9.0 – 9.9	631	243		
10.0 – 10.9	317	91		
11.0 – 11.9	247	89		
12.0 – 12.9	178	54	30.4	11.7†
13.0 – 13.9	112	51		
14.0 – 14.9	66	19		
Over – 15.0	224	85		
Totals	8,847	8,847	100.0	100.0

* These 8,847 sera are 53.5% of the total originally 5.0 T.T. units or more.

** Change in T.T. unit value: Average: 3.7 T.T. units lower. Range: from 2 higher to 10 T.T. units lower.

† When those without positive flocculation are excluded the "abnormal" sera over 8.0 T.T. units approximate 2%.

or more with positive T.F. in lipemic sera (98.3% of the tests), were both used as the screening levels for the study of the incidence of apparent transfusion-caused serum hepatitis in patients receiving these bloods.[1] It was believed that comparison of the results with and without inclusion of the equivocal test area (+ 2 to + 3 S.D.) would be valuable, because the results ought to be more clear cut and reliable when only definitely abnormal test results are considered. Of the total 52,662 bloods, 5.4 per cent (2,824) could be considered "positive" at the T.T. 8.0 unit and over range (95% confidence) for screening purposes and, at the 10.1 unit level (99% confidence), there were only 1.7 per cent "positive," as shown in Table 4.

The Effect of Inactivation of the Test Sera: During the first year of the study, a series of about 80 donor sera which had been inactivated for the VDRL test (incubation at 56 C. for 30 minutes), were accidentally used for the T.T. test. The mistake was detected at once because the distribution of T.T. values seemed to be too low in comparison with past experience. Repetition of this test series with the original (uninactivated) sera confirmed this. During the remainder of the first year of the study, normal and inactivated sera readings were recorded for several hundred test sera to attempt to characterize and evaluate the changes in the test results. Although considerable inquiry and study failed to provide an adequate explanation, it became apparent that, if the results were evaluated on the same tentative screening criteria (8.0 to 9.3 T.T. units[2, 4]) which we assumed correctly would prove close to our calculated level of significance, the percentage of "abnormal" tests by this modified method much more closely approximated the reported incidence of less than one per cent hepatitis carriers[5] in the population and it was felt worthwhile to study this further for possible significance. Therefore, during the second year of the study, all sera with T.T. values of more than 5.0 units were retested after inactivation. The results are summarized in Table 5. There were 8,847 such sera, 53.5 per cent of the total

number found to be 5 T.T. units and over during the entire study. The "rough" mean (sera under 5 T.T. units were not tested) for this method for clear sera was found to be 2.7 T.T. units. However, this was determined after including the sera of 5 T.T. units or less which were tested during the same period, with the T.T. values reduced to a slightly lower figure, based upon our original observations of the several hundred sera tested both ways. The standard deviation was found to be 2.1, which indicated the screening level of probable significance (+ 2 S.D.) was 6.9 T.T. units and 9.0 units for definite abnormality (+ 3 S.D.), considerably lower than the results on unheated serum. The high variations observed between the two tests at each level of original T.T. value accounts for the high standard deviation. Total inactivated sera T.T. values were an average of 3.7 units lower, and varied from a few with slightly higher values (1 to 2 units) to a few lowered as much as ten T.T. units. We still have no adequate explanation for this other than the obvious: that inactivation in some way alters the lipoprotein fractions of the serum.

Discussion

The wide variation in the results of the thymol turbidity test in 52,622 consecutive blood donors who were normal and healthy enough to be accepted, according to nationally approved standards, merits some comment. The test, ordinarily employed as a diagnostic aid in patients with known or suspected liver disease, is stated[8] to have an upper limit of 5 Shank-Hoagland units at *p*H 7.55 in "normal" fasting individuals and an upper limit of 8 units in nonfasting persons. Although Neefe and associates[7] found, in a control series of normal, nonfasting, young adults, a mean of 3.3 Shank-Hoagland units, a + 2 S.D. value of 5.5 and + 3 S.D. value of 6.6 T.T. units, the present study shows a mean of 3.8 T.T. units, a + 2 S.D. of 8.0 and + 3 S.D. of 10.1 T.T. units in a very large series of apparently healthy, adult blood donors (45,922 were nonlipemic sera). It is noteworthy that the only other similar study[4] of blood donors which has been reported, and in one other study of nonfasting adults,[8] the results very closely approximate ours. However, it was not stated whether either included both clear and lipemic (turbid) sera. Because lipemia does cause false positive T.T. tests, it seemed essential to us that high T.T. results in such sera be confirmed by the presence of flocculation[6, 8] and that they be excluded in computing the mean and standard deviation to establish the criteria of abnormality.

It must be emphasized that these individuals represent a good cross section of the healthy working population. Based on a review of over three thousand consecutive donors, their age distribution is approximately: 4 per cent from 18 to 20, 31 per cent from 21 to 29, 34 per cent from 30 to 39, 24 per cent from 40 to 49, and 7 per cent from 50 to 59. They have the following reasonably representative, occupational distribution:[3] Craftsmen, 21 per cent; Laborers, 19 per cent; Armed Services, 14 per cent; Operatives, 14 per cent; Service Workers, 10 per cent; Professional Technical, 5 per cent; Sales, 4 per cent; Clerical, 3 per cent; Housewives, Managers, Students, Foremen, and Farm Laborers, about 2 per cent each. They represent a quite stable population group, for 72 per cent are permanent residents of the community when they serve as blood donors and many give blood repeatedly. In our opinion, it is quite possible that, in this large group, the 1.7 per cent of blood units with "definitely abnormal" T.T. values (by the + 3 S.D. criteria used in our series) were drawn from individuals (about 370) who have had in the past some illness (viral) or toxic insult (drugs, alcohol) which has left them with residual liver disease and a truly abnormal

thymol turbidity test. It is considered less likely that the 5.4 per cent "abnormal" at the + 2 S.D. (equivocal) level (about 1,200 persons) actually had significant liver disease. Since the usual method of establishing the "normal" range of a clinical laboratory test, as well as the significant "abnormal" values, is to test a group of normal, young, healthy persons, one wonders whether this method may not usually produce "ideal" rather than "practical" levels which can intelligently be applied to a cross section of the adult population. Perhaps, the use of blood donors for such purposes has a very real and practical value.

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Thymol Turbidity and Thymol Flocculation Tests As a Screening Technic to Detect Blood Donors Carrying the Hepatitis Virus:*

II. The Results of Follow Up Studies of Six Months or More on 2,385 Transfused Patients

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Six-month follow up study showed a hepatitis case rate of 1.55% in 905 patients receiving "abnormal" blood and 0.88% in 1,480 receiving nothing but "normal" blood, based on screening at the 95% confidence level of 8.0 T.T. units in clear sera and in lipemic sera of 8.0 units or more with a positive flocculation test (from studies of 52,662 consecutive donors reported in Part I). Significantly, screening at the 99% level gave less specific results: a 1.06% rate in controls and 1.42% in the test cases. The observed differences in rates *are not* statistically significant: twice the standard error of the difference in percentages is 0.9%, the Chi Square test showed P = 0.15 (at 95%) and 0.70 (at 99%). The donor (unit) case rates were essentially identical (0.346) in the test series and in the controls at both screening levels and the difference in hepatitis case rates was exactly proportional to the difference in the average donor exposure of the two groups at each screening level. The two groups of cases received 14% of the total blood units available and this was representative of the total available as judged by the mean T.T. values of blood received by the study cases. There was a steady increase in the hepatitis case rate from 0.6% in single unit transfusions (29% of cases) to 2.6% when four units were used, with a rate of 1.1% in those receiving five units or more (19%), suggesting the risk of hepatitis is maximal in transfusions of four or more units. Cogent reasons are presented for abandoning attempts to use nonspecific liver function tests for donor screening to eliminate significant numbers of donor carriers.

CONFLICTING reports have appeared in the literature during the past several years regarding the efficacy of one or more of the liver function tests in detecting a significant number of donors who may be carriers of the hepatitis virus. The most frequently used has been the thymol turbidity test (T.T.) because of its relative low cost and simplicity. Screening has been done both at the 95 per cent confidence level (+ 2 Standard Deviations) and at the 99 per cent confidence level (+ 3 Standard Deviations). Neefe and co-workers,[11] Jennings and co-workers,[5] Strumia and co-workers,[14] and Norris and co-workers[12] all have suggested that the use of a T.T. screening level appreciably above normal will be of significant value (statistically valid) in detecting prospective blood donors who are virus carriers. Contrariwise, Loder,[7] Rosenberg,[13] Katz and associates,[6] and, recently, Maxwell[8] have not been able to demonstrate the usefulness of the T.T. test for this purpose. (Both Strumia and Katz also used other liver function tests in addition to the T.T. test in arriving at their conclusions.) Several of these reports have been discussed in more detail in a previous publication.[1] More recently, Bang and associates[3] reported evidence from which they conclude that elevated serum transaminase (SGOT) levels are present in a significant percentage of hepatitis virus carriers, and Bowers and co-workers[4] have suggested that the same relationship may hold when elevated levels of serum isocitric dehydrogenase activity (ICD) are present in prospective donors.

Because the risk of the accidental transmission of serum hepatitis is one of the most serious problems today in blood

* Received for publication March 4, 1961; accepted June 3, 1961.

transfusion therapy, and because its continued occurrence is a frequent source of legal action against physicians, hospitals and blood banks, it seemed important to study further the possibility that a liver function test could aid significantly in the prevention of this hazard. Therefore, this study was undertaken in December 1957. The screening of donor bloods was discontinued in January 1960 and the patient follow up studies were completed in December 1960.

Method

Results of the Thymol Turbidity and Flocculation Screening of the donor bloods used in this study has been presented in detail elsewhere.[2] The screening level of significant abnormality for clear sera was found to be 8.0 T.T. units or more at the 95 per cent confidence level (equivocal) and 10.1 T.T. units at the 99 per cent confidence level (definitely abnormal). As previously pointed out, high T.T. values in turbid sera are often false positives.[2] Therefore, only lipemic sera of 8.0 units or more and 10.1 T.T. units or more, respectively, with a positive thymol flocculation test (T.F.) were considered significantly abnormal. The "abnormal" bloods comprised 5.4 per cent of the total tested at the 95 per cent confidence level, and 1.7 per cent at the 99 per cent level.

Initial (Tentative) Selection of Patients for Follow Up Study was accomplished in the following manner. All bloods with "abnormal" test values of 8.0 T.T. units or more were listed and their use was traced. Every tenth blood with "normal" test values was also listed and traced. If an "abnormal" blood was used in a local (Phoenix) hospital, this patient was then listed for follow up six months later, and two patients in local hospitals, who had received "normal" blood collected at about the same time, were selected as controls. The selection of twice as many control patients was done in an effort to obtain a more equitable proportion of followed cases to total cases in the two groups: (a) patients receiving "abnormal" blood, the test group, and (b) patients receiving nothing but "normal" blood, the control group. Since it is well known that the actual number of hepatitis cases which occur and will be found on follow up is much greater than the number voluntarily reported, and since many selected would have to be excluded later because they would also receive "unknown" units, or would shift from the control to the test series; the follow up of equal numbers in the two groups could easily "load" the study by chance, because only a relatively small per cent of patients receiving transfusions would receive "abnormal" blood (at the 95 per cent confidence level only 5.4 per cent of the total blood units were "abnormal").

Interim Procedure: Each selected patient was listed and followed in respect to identification of all other units of blood or blood derivatives received, both for several months prior to the "key" unit and during the ensuing six-month period. All such units were listed on each "case" card.

Final Selection: At the end of six months, the "case" cards were reviewed and either accepted or rejected for follow up study. All cases receiving one or more units of "abnormal" blood were accepted for the test group, and all cases who received nothing but blood found to be "normal" by the T.T. (or T.F.) test were accepted for the control group. Once a month, the names of the patients approved for follow up were sent to the hospital where the blood had been administered to obtain the name of the patient's physician and the patient's home address.

Follow Up of Selected Cases: Each patient's physician was then contacted and requested to complete a "follow up questionnaire" specifically concerning possible viral hepatitis. Whenever no report was received after a reasonable period, or when

the physician's contact with the patient was for a period of less than six months, the case was referred to the Visiting Nurse Service for a home call. The nurses obtained the necessary information indirectly under the guise of a "posthospitalization health survey."

As the acceptable follow up reports (six months or more) were received from either source, the "case" card was reviewed: (1) in negative reports (no viral hepatitis) all blood units in the study administered up to six months before the date the patient was last seen by the physician or contacted by the Visiting Nurse Service were included; (2) in positive reports (viral hepatitis) all study bloods used up to 40 days prior to follow up had to be included routinely as possible sources. All others were excluded from consideration, unless a "short incubation" serum hepatitis[9] could be verified.

Criteria for the Diagnosis of homologous serum jaundice, presumably due to blood transfusion, were as follows: (1) The onset of symptoms (usually jaundice) must have occurred between the 40th and 180th day after the date (s) of administration of the blood unit (s) apparently responsible; (2) if the patient was hospitalized and/or studied by a clinical laboratory, the results of appropriate liver function tests* must be compatible with the diagnosis of viral hepatitis; (3) if the patient was treated entirely at home, confirmation of viral hepatitis was based on the symptoms, severity and duration of the hepatitis and the absence of any other apparent cause; and (4) there must have been no evident exposure to infectious hepatitis within the previous 15 to 40 days.

Criterion (1), above, requires comment. Although the usually accepted incubation period for serum hepatitis is 60 to 160 days,[15] we believe that both shorter and longer incubation does occur occasionally with the SH virus and that it was, therefore, reasonable to accept a lower limit of 40 days, the maximum incubation period generally accepted for the infectious (epidemic) type,[15] and an upper limit of 180 days. While it is recognized that short incubation serum hepatitis (less than 40 days) may occur after transfusion,[9] presumably transmitted by donors who are carriers of the IH virus A rather than the SH virus B, we did not feel justified in accepting a lower limit of less than 40 days for routine use. Our experience in the follow up of more than 150 reported cases has convinced us that an incubation period of less than 40 days post-transfusion is quite rare in patients with typical, acute viral disease of the liver, including adequate laboratory studies (especially the transaminase), who have not had known exposure to infectious hepatitis within the previous 15 to 40 days. All reported cases are studied as carefully as circumstances permit. When the diagnosis is clear, or when lack of adequate information leaves it in doubt, the short incubation case is considered to be possibly transfusion caused.

The study was carried out over a period of nearly three years and the splendid cooperation received throughout from the hospitals, the physicians, and the Visiting Nurse Service is directly responsible for the success of the follow up program.

Results

Of the 3,942 cases available for six-month follow up, it was possible to trace 2,771 (70%), of whom 386 (16% of those followed) had expired prior to the six-month period, leaving 2,385 cases with a satisfactory follow up. Because of the large numbers of tourists and itinerant farm laborers in the Phoenix area, it is not surprising that 1,171 (30%) of the cases selected had left the area and could not be traced. Table 1 shows the details of blood usage by the 2,385 followed patients and Table 2 shows the serum hepatitis case rates found in the

* Preferred tests were: serum transaminase, direct and indirect serum bilirubin, thymol turbidity, and cephalin flocculation.

TABLE 1. *Blood Usage in the Followed Cases at the 95% and 99% Screening Levels*

Followed cases		Number of cases	Total blood units used	Average number of units used	Total "Abnormal" T.T. units used	Average "Abnormal" T.T. units used	Per cent of total units (52,662) used in study cases
Control	95%	1,480	3,743	2.53	—	—	7.1
	99%	1,964	6,080	3.09	—	—	11.3
"Abnormal"	95%	905	4,038	4.46	1,041	1.15	7.7
	99%	421	1,701	4.04	477	1.13	3.5
Totals		2,385	7,781	3.26	—	—	14.8

two groups of cases. Both tables show results at the 95 per cent and 99 per cent confidence screening levels. It will be noted that there are, at the 95 per cent confidence level, 1,480 control cases (all units of known "normal" blood) and 905 test cases, or 38 per cent of the total (one or more units of "abnormal" blood). The average use of blood by the 905 test cases (4.46 units) was almost twice as much as used by the control series (2.53 units), but each group used about the same percentage of the total bloods issued for use during the study period, amounting together to 14.8 per cent of the blood. At the 99 per cent screening level, there are 1,964 control cases and 421 test cases (18% of the total), the average blood use (3.09 and 4.04, respectively) becomes more similar, but the test cases used only 3.5 per cent of the blood. Note that, although the donor exposure of controls and test cases differs more at the lower than the higher screening level, the exposure to "abnormal" bloods does not.

At the 95 per cent confidence level, there were 13 cases of hepatitis found in the control group, a case rate of 0.88 per cent, and 14 cases found in the test group, a case rate of 1.55 per cent. The rate for the entire group of 2,385 followed cases was 1.13 per cent. Analysis at the 99 per cent confidence level is shown in the last two columns of Table 2. The less significant results, a control case rate of 1.06 per cent and a test case rate of 1.42 per cent, are noteworthy and demonstrate the paradoxical results when the equivocal test results are excluded and only the definitely abnormal are used. These results are quite comparable to, although slightly higher than, the rates Maxwell[8] reported in followed cases at the 95 per cent confidence level (0.68% in controls and 0.69% in test cases).

All of these 27 cases met the criteria for

TABLE 2. *The Incidence of Serum Hepatitis*

Followed cases	95% Confidence Level			99% Confidence Level		
	Number of cases	Serum hepatitis	Case rate*	Number of cases	Serum hepatitis	Case rate*
Control	1,480	13	0.88%	1,964	21	1.06%
"Abnormal" T.T.	905	14	1.55%	421	6	1.42%
Totals	2,385	27	1.13%	2,385	27	1.13%

* Standard Error of Difference in Rates, significant difference is more than 0.9%.
Chi square, P = 0.15 for 95% Confidence Level; P = 0.70 for 99% Level.

serum hepatitis previously described. It is of some interest to point out that, in spite of widespread knowledge of the study among the area physicians, only eight of the cases were voluntarily reported, 14 were reported by physicians on receipt of the follow up questionnaire, and five were found later through the home calls made by the Visiting Nurse Service. In this last group of cases, the original physician had lost contact with the patient and another physician was consulted for care of the hepatitis illness. In each of these instances, it proved possible to obtain verifying laboratory and other data from either the attending physician or the hospital pathologist. There were no cases of verified "short incubation" serum hepatitis, although infectious hepatitis was endemic in the area during the study. At the 8.0 T.T. level, there were 97 cases who received two "abnormal" units with two cases of hepatitis, 15 received three with one case, and three received four with no cases. At the 10.1 T.T. level, there was one case of hepatitis in 35 receiving more than one "abnormal" unit.

The higher incidence of serum hepatitis observed in the cases receiving "abnormal" T.T. blood *is not* statistically significant at either screening level and, in fact, is almost exactly proportional to the observed difference in the donor exposure of the two groups at both screening levels. The standard error of the difference between the case rates observed is 0.45 per cent, therefore, a significant difference would have to be greater than 0.9 per cent. The observed difference is 0.67 per cent at the 95 per cent level and only 0.36 per cent at the 99 per cent level. The Chi square test of significance at the 95 per cent level shows that P = 0.15 and at the 99 per cent level P = 0.70. The generally accepted criteria of significance in the Chi square test are that P must be less than 0.01 for a positive conclusion of validity, that between 0.01 and 0.05 results may be significant, and that P values of over 0.05 indicate that the observed difference is not significant. The donor case rate ("carrier" donors) is 0.346% (one in 288 donors), as shown in Table 3, and differs by only 0.001 per cent in the control and test groups at the 95 per cent screening level and not at all at the 99 per cent level. The true donor carrier rate probably approaches 1 per cent, because immunity is known to exist in some 50 to 60 per cent of the population.[9] The donor case rate is based on the belief that presumably only one of the bloods used in each case of serum hepatitis transmitted the virus responsible for the disease.

It is of interest to compare the two groups further. Table 4 shows the mean values of the T.T. test results both at the 95 per cent and 99 per cent screening levels for the bloods used by the control group, the test group, the cases of hepatitis in each group, and for all bloods available during the study. The mean values for all bloods in-

TABLE 3. *The Donor Case Rates*

Followed cases	95% Screening Level			99% Screening Level		
	Number of donors (units used)	Serum hepatitis	Donor (unit) case rate*	Number of donors (units used)	Serum hepatitis	Donor (unit) case rate*
Control	3,746	13	0.347%	6,079	21	0.346%
"Abnormal"	4,038	14	0.346%	1,705	6	0.346%
Totals	7,784	27	0.346%	7,784	27	0.346%

* Standard Error of Difference in Rates, significant difference is more than 0.26%.

TABLE 4. *The Mean T.T. Values of Bloods Used in the Study Cases at the 95% and 99% Screening Levels*

Followed cases		2,385 Total Cases (7,784 units)		27 Hepatitis Cases (104 units)		Total Available Blood (52,662 units)	
		Mean T.T. all blood	Mean T.T. nonlipemic blood	Mean T.T. all blood	Mean T.T. nonlipemic blood	Mean T.T. all blood	Mean T.T. nonlipemic blood
Control	95%	4.0	3.5	4.7	3.8	—	—
	99%	4.5	3.9	4.8	3.8	—	—
"Abnormal"	95%	6.0	4.7	6.2	4.1	—	—
	99%	6.6	5.1	7.6	5.1	—	—
Totals		5.0	4.3	5.6	4.0	4.4	3.8

volved and the mean values for the nonlipemic bloods only are tabulated for each group. The differences certainly are not remarkable at the 95 per cent screening level, since somewhat higher mean values would be expected in the test cases (receiving 21% of "abnormal" blood). On the other hand, there is an apparently real difference at the 99 per cent level, especially in the group of hepatitis cases in the "abnormal" series. However, it seems evident from the total figures that the followed cases received a representative 14.8 per cent of the total blood available during the study period and that the 27 cases of hepatitis received bloods which were not appreciably different from those given to others in each study group.

Table 5 shows the distribution of cases, per units transfused at the 95 per cent screening level. Single unit transfusions were given to 35.5 per cent of control cases and 19.4 per cent of the test cases, a total of 29.4 per cent in the study. Two to four unit transfusions were received by 51.3 per cent of the study group. Five or more units were received by 19.3 per cent. (At the 99 per cent screening level only 16 per cent of the test cases received single units and 68 per cent five or more. The six hepatitis cases received 1, 2, 3, 4, and 8 units of blood, respectively.) The number of cases

TABLE 5. *Distribution of Cases per Units Transfused*

Number of units transfused	% of control cases	Number of hepatitis cases	% of "Abnormal" cases	Number of hepatitis cases	% of total cases	Total number of hepatitis cases
1	35.5	2	19.4	2	29.4	4
2	29.2	2	22.2	5	26.5	7
3	14.9	4	15.3	1	15.0	5
4	8.2	3	12.5	3	9.8	6
5	4.8	1	7.5	0	5.8	1
6 – 10	6.5	1	15.4	1	9.9	2
11 – 15	0.9	—	5.2	0	2.6	0
16 – 20	—	—	1.2	2	0.5	2
20 – 25	—	—	0.6	—	0.2	—
Over 25	—	—	0.7	—	0.3	—

TABLE 6. *Other Liver Disease*

Diagnosis	Control series	Test series	Total
Toxic (drug) hepatitis	2	1	3
Carcinoma of the liver	0	2	2
Cirrhosis of the liver	2	0	2
Common duct stone	1	0	1
Hepatitis, non-viral (diagnosis confirmed; 30 days post-transfusion)	0	1	1
Hepatitis, viral, infectious (epidemic) (diagnosis confirmed; 19 days post-transfusion)	0	1	1
Total	5	5	10

per units transfused is not large enough to compare hepatitis case rates in the test groups with the corresponding control groups. However, in the total study group, 22 of the 27 cases of hepatitis occurred in the 1,925 patients (80.7%) who received one to four units, there being five cases in the remaining 460 patients (19.3%) receiving five units or more. Interestingly, there is a steadily increasing incidence in these larger total groups of cases receiving one through four units (0.6%, 1.1%, 1.4% and 2.6%, respectively). Although the case groups are small and the hepatitis cases few, the rate for all cases receiving five units or more is, in fact, slightly lower (1.09 *versus* 1.14%), in spite of the increasing donor exposure. This suggests that the critical point in an increasing donor exposure *versus* an increasing risk of hepatitis may lie at about the level of four units of blood.

Other forms of liver disease were observed in ten of the followed cases, as shown in Table 6. There were five such cases in each series of patients, including two cases in which possible SH with "short incubation" periods of 19 and 30 days had to be ruled out. After careful study, the case of non-infectious hepatitis in the high T.T. series could not be considered as possible serum hepatitis because the clinical course and the liver function tests were not compatible with acute viral hepatitis, although the blood received was "definitely abnormal." The one case of typical viral hepatitis occurring in the high T.T. series was diagnosed as Infectious (Epidemic) Viral Hepatitis because of the positive history of exposure 25 days prior to onset. This case received blood with an "equivocal" T.T. test result.

In addition to the cases of hepatitis in the study group, there were 18 cases of apparent transfusion-caused viral hepatitis reported voluntarily among the transfused patients not selected for follow up. A total of 43,878 units of blood was used in the Phoenix area during this period for these patients. Based on the average use of blood by the study group, these units were administered to approximately 13,460 patients, a case rate of 0.13 per cent—slightly more than one-tenth of the rate of 1.13 per cent in the followed group, which compares favorably with Maxwell's[8] observations. Four of these 18 cases received only "unknown" units (from other banks—no T.T. test); seven received "known" thymol units (all "normal") plus one or more "unknowns"; and seven received nothing but "known" thymol units. Only one of these last seven cases (all "known" units) occurred in a patient receiving T.T. blood which was in the "equivocal" T.T. test range.

The Results with Heated Sera,[2] as expected from analysis of the test results, showed no improvement in specificity over the data just reported. Re-sorting of the followed cases, based on the 6.9 T.T. or more (95% confidence level) screening value established for the test on inactivated clear sera and 6.9 T.T. or more with positive flocculation on inactivated lipemic sera, gave a control group of 1,882 cases with 20 cases of hepatitis (case rate 1.06%) and 455 test cases with five cases of hepatitis (case rate 1.08%), and a rate for the total group of 1.07 per cent. Forty-eight of the original test cases (including two of the hepatitis cases) had to be discarded because all bloods "abnormal" to the standard T.T. test had not been retested by this method.

Discussion

The results reported here substantiate the recent work of Maxwell[8] and earlier studies by Loder,[7] Rosenberg,[13] Katz, *et al.*,[6] and confirm their conclusion that the thymol turbidity test is of *no value* in identifying a *significant* number of virus carriers among blood donors. The significant data were presented and evaluated at both the 95 per cent confidence level (+ 2 S.D.) and at the 99 per cent confidence level (+ 3 S.D.) because studies in the literature[8, 11, 12] have used both criteria. However, these levels were determined on clear sera, because lipemia often causes false positive results.[2] Lipemic sera were considered abnormal only if the T.F. test was also positive. The fact that the results obtained at the 99 per cent level of positive abnormality were poorer than when the equivocal tests (+ 2 to 3 S.D.) were also included is paradoxical and is believed to be of considerable import. One would expect that the reverse would be the case in a test of significant value in the detection of donor carriers.

We feel entirely justified in not including the two cases in Table 6 which might have been considered as "possible" serum hepatitis because a different diagnosis was made, based on careful clinical and laboratory studies. The case of infectious hepatitis received three units of blood, one of which was "abnormal" at the 8.0 T.T. unit screening level, and this case was one of many occurring in the area at that particular time. The other case, non-viral hepatitis, received a single unit, "abnormal" at the 10.1 T.T. screening level. In any event, inclusion of these cases at the 95 per cent screening level would not quite reduce the Chi square P value below the significant level of 0.05, nor increase the difference in rates to a significant value. There would be no change at the 99 per cent screening level. It should also be emphasized that there are seven cases among the 18 reported outside of the study group, six of whom received only known "normal" thymol blood and one known "abnormal" blood, but with an "equivocal" test result. These should be considered as well as the two cases just discussed in fully evaluating the report, for they add some weight even though they were not selected for the follow up study.

It is of interest that the incidence rates which we observed in the followed cases, although slightly higher, rather closely approximate those found by Maxwell.[8] The fact that he did not employ the T.F. test to validate high T.T. results in lipemic sera as we did perhaps accounts for the higher case rate in our test series, since this reduced the number of followed cases receiving "abnormal" blood. The similarity also exists in the voluntarily reported case rates in the patients who were not followed in both studies, our findings again being somewhat higher. Both of these studies serve to emphasize the common observation that voluntary reporting is usually poor in diseases where isolated cases are not ordinarily a particular hazard to others. Unfortunately, this makes it more difficult for blood banks to protect patients against donors who might thereby be excluded as "suspect carriers" of the virus by biologic

criteria* previously described.[1] In our opinion, the evidence here presented, together with the studies by Maxwell[8] and others,[6, 7, 13] far outweighs the evidence presented by Neefe,[11] Jennings,[5] and Strumia,[14] which led them to recommend that the use of these tests would significantly reduce the incidence of hepatitis.

The National Cancer Institute[10] published a pertinent bulletin on the principles and criteria for the evaluation of screening tests for the early detection of cancer in 1950. It seems to us high time that similar, sound criteria of validity be applied by workers who evaluate tests of various sorts employed in an effort to screen out blood donors who are likely to be carriers of the hepatitis virus. The two situations are quite similar in that the cancer screening test is not diagnostic for cancer, but must be rather specific, and a positive test must be confirmed by appropriate diagnostic methods. In other words, such a test for donor screening should also be reasonably specific for the carrier state rather than a nonspecific measure of liver function, which bears no such direct relationship. These criteria of specificity were developed after a careful statistical study of the requirements for an acceptable screening test for cancer. To summarize, a test will be considered valid and become acceptable for use for this purpose, if it is sufficiently specific that: (1) it will be positive in at least 90 per cent of patients subsequently found to have early cancer, and (2) there will be less than 5 per cent of false positive results. Let us apply these excellent criteria of specificity to our attempt to employ the presence of significantly abnormal thymol turbidity levels to screen out blood donors who are virus carriers. At the + 2 S.D. level, the T.T. test was positive in one or more units of blood used by only 52 per cent (14 of 27) of the followed cases who acquired serum hepatitis. It was also positive in one or more units of blood used by 891 patients who did not get the disease—98.6 per cent false positive results. At the + 3 S.D. (99%) level, the test was even less discriminatory; there were but 22.2 per cent (6 of 27) correct positives and 98.6 per cent (415) false positives. This seems to us such poor accuracy as to make the use of the thymol turbidity test unreasonable and unacceptable for the screening of blood donors.†

The thymol turbidity test and all other available chemical tests of liver function are nonspecific and are designed only to detect a malfunction of the liver as an aid in the differential diagnosis of patients with clinically evident or suspect liver disease. Since by no means the majority of carriers have abnormal test results, none of the available tests can be used successfully, either singly or in combination, to detect the presence of this specific entity, the presence of the hepatitis virus in a prospective donor. Let us stop trying to use such tests for a function they can not perform, thereby continuing to delude many responsible for the collection and administration of blood (and the public), and to add strength to the case of the plaintiff who sues because of alleged transfusion-caused serum hepatitis.

* These provide that a suspect donor is excluded unless: 1) he can be recontacted to confirm the negative history concerning the possibility of earlier virus hepatitis or exposure to it, 2) he has on record with us at least two prior donations within the previous six to 18 months which did not result in presumed transfusion-caused virus hepatitis in the patient, and 3) he is not the only donor involved (single unit transfusion) or is a second "offender."

† To satisfy ourselves that we were not being too arbitrary concerning a test which eliminated about one half of the cases of apparent transfusion-caused serum hepatitis at a screening level of + 2 S.D. (95%), we indulged in an exercise employing nonscientific donor criteria to evaluate this series of cases. The results were not unexpected. Donors in the 30 to 39-year age group were associated with 66 per cent of the cases, those whose first initial was "J" or "R" with 61 per cent, and, when the sum of the last two digits of the blood (donor) unit numbers was "11" or "14," these donors were involved in 50 per cent of the cases. These percentages were all much higher than their normal occurrence (34, 28 and 13%, respectively) in a series of over 3,000 donors.

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Notation for the Kell Blood-Group System*

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THE RECENT discovery of several new types within the Kell blood-group system has suggested that it may prove to be as complex as are the Rh and MNS systems. The present chaotic condition of nomenclature in the Rh and MNS systems suggests the desirability of choosing a better notation for Kell while this can still be done with ease. The following notation, adapted from the Salmonella classification of Kauffmann,[4] is proposed. It is similar to a notation for Rh proposed by Rosenfield and Allen.[6] Numbers are used instead of letters for each qualitatively different kind of antiserum that defines the presence or absence of a serological property inherited as part of the Kell blood-group system. It is believed that numbers are less likely than letters to imply an unproven antigenic determinant or an unsupported sub-locus within a complex gene. The term "antigenic determinant" refers to a chemical structural grouping, on an antigen, which (1) directs the specificity of antibody incited by the antigen, and (2) reacts with the specific combining site on the antibody molecule. A "sub-locus within a complex gene" refers to a hypothetical site of mutation responsible for a single antigenic determinant. There is no proof that serological reactions define single antigenic determinants. Antigenic determinants can be defined precisely only by elucidation of their chemical structure.

Aided by Grant H-4456, National Heart Institute, U. S. Public Health Service, and by the A. A. List, Frederick Machlin, and Anna Ruth Lowenberg Research Funds.

* Received for publication April 28, 1961; accepted June 15, 1961.

Phenotypes

Table 1 shows the Kell phenotypes now known. All but the last have been published previously.[5] McLeod is described in a separate communication.[1] The original names (Kell, Cellano, Penney, etc.) that were used for antiserums of the Kell system are retained, but are given also a numerical designation in the order of their discovery. Kell is K1, Cellano is K2, etc. K5, detected by the Peltz serum, was originally termed "Ku."[3] The short names (*e.g.* Kell+) for phenotypes will undoubtedly be used in active laboratories, as they have in the past. The full names are taken from the observed reactions with the five known reagents. The letter K is followed by a colon, then by the numbers corresponding to the antibodies that are used in testing. To show that no reaction is observed, a minus sign is inserted before the number, and a weak variant is indicated by preceding the number with the letter "w."

If only anti-K1 is used in testing, the results are designated K:1 or K:−1 (or Kell+, Kell−). Similar use of the other terms, K2 to K5, singly or in combinations, may be employed as desired or needed. This permits latitude in designating the results of whatever tests are done.

Genes

In Table 2 are shown the designations for the complex genes. The basic symbol, *K*, is always italicized, but is capitalized only when the gene determines one or more recognizable antigenic determinants. A lower case letter, *k*, is employed only to designate the amorphic gene, which produces none of the known antigenic deter-

TABLE 1. *Known Phenotypes of the Kell Blood-Group System—1960*

Short name	Full name	Antigenic structure of phenotypes as shown by reactions with various antiserums: K1 Kell	K2 Cellano	K3 Penney	K4 Rautenberg	K5 Peltz	Approximate frequency
Kell+	K:1,2,–3,4,5	+	+	0	+	+	.09
Kell–	K:–1,2,–3,4,5	0	+	0	+	+	.88
Cellano–	K:1,–2,–3,4,5	+	0	0	+	+	.0025
Penney+	K:–1,2,3,4,5	0	+	+	+	+	.02
Kell+, Penney+	K:1,w2,3,4,5	+	weak	+	+	+	.004
Rautenberg–	K:–1,w2,3,–4,5	0	weak	+	0	+	.0002
Peltz type	K:–1,–2,–3,–4,–5	0	0	0	0	0	.0001
McLeod type	K:–1,w2,–3,w4,–5	0	weak	0	weak	0	.0001

minants of the Kell series. Previous blood group notations have failed to change lower case letters after the discovery of antigenic determinants produced by genes so designated, and this has led to much confusion. This corrected use of the symbol *k* should not be a serious source of confusion if, in new papers for the next few years, authors include a brief table defining the terms. Genes other than *k* are characterized by superscript numbers showing the antigenic determinants produced, or not produced, by them.

With the exception of the uncommon gene *k*, Kell genes produce either K1 or K2. Thus far there is no evidence of the existence of a gene that produces both K1 and K2, though the data are scanty and mostly statistical in nature. (There is no excess of K:1,2 children from matings in which both parents are K:1,2.) Similarly, all genes except for *k* produce either K3 or K4, but (so far as known) no gene produces both.

Gene *k* produces none of the known antigenic determinants of the Kell series. Mrs. Peltz, of the rare homozygous genotype *kk*, developed anti-K5, which indicates that the gene *k* does not produce K5. No antigenic determinant has been found that has the same reciprocal relation to K5 that K1 and K2 have to each other. K5 is extremely common (± .9999); therefore, an alternative antigenic determinant would be expected to be uncommon (± .02, or possibly much less frequent). Each of the uncommon antigenic determinants so far described has been shown not to be the alternate of K5. This constitutes part of the evidence for considering gene *k* to be an amorph.

TABLE 2. *Allelic Genes of the Kell Blood-Group System—1960*

Full name	Gene products as revealed by serological reactions observed in known homozygotes: K1 Kell	K2 Cellano	K3 Penney	K4 Rautenberg	K5 Peltz	Approximate frequency
$K^{1,-2,-3,4,5}$	+	0	0	+	+	.045
$K^{-1,2,-3,4,5}$	0	+	0	+	+	±.935
$K^{-1,w2,3,-4,5}$	0	weak	+	0	+	.01
k	0	0	0	0	0	±.01

All the well-studied Kell genes except *k* produce K5, though there are unpublished data,[2] in addition to that on the blood of Mr. McLeod (Table 1), suggesting the existence of genes besides *k* that do not.

It is evident that anti-K5 is not needed to differentiate any of the present Kell phenotypes or alleles, but it may be of value in the future. Thus K:1,–2,–3,4 means the same now as does K:1,–2,–3,4,5, but the term K:1,–2,–3,4 indicates that anti-K5 was not employed.

Interpretation of an Alternative Theory

Race and Sanger[5] mention an alternative interpretation of the facts, which assumes that one type of Kell gene produces only K1, a second allelic gene produces only K2, a third allele produces only K3, and a rare recessive amorph produces no known antigenic determinant. Anti-K4 of Table 2 is considered by this theory to consist of antibody molecules having one specific combining site for K1 and another specific combining site for K2 (for which there is no supporting data nor proven immunological analogy), or a cross-reaction (anti-K1 that cross-reacts with K2). Anti-K5 of Table 2 is also considered to be a cross-reaction: this, too, lacks supporting data and fails to recognize the structural groupings responsible for cross-reactions. To explain the reaction of anti-K2 with phenotype K:1,w2,3,4,5, it might be postulated that anti-K2 cross-reacts with K3. Unfortunately, this theory is not subject to proof, though it might be disproved if incorrect. Actually, recent developments[1, 2] provide some evidence against it, three bloods having now been found (including McLeod, shown in Table 1), that apparently lack K5 while having both K2 and K4.

Summary

A new notation for the Kell blood-group system is presented, based on numbers instead of the familiar letters. It is hoped that this notation will be adopted to help determine whether a purely numerical system would be suitable for use with the more complicated Rh and MNS systems. It is suggested that when the new notation is used in new publications, a brief statement of the equivalent terms in the old and new notations be made at the beginning.

Acknowledgment

We are grateful to Dr. Bruce Chown, Dr. Shaul Kochwa, Dr. William C. Boyd and Dr. Elvin Kabat for helpful criticism and advice.

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Principles of Blood Group Serology and Nomenclature:*

A Critical Review

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The A-B-O group system of man is used as a model to explain the principles of blood group serology and nomenclature. A correct nomenclature must take into account three kinds of entities, genes, the gene products (agglutinogens or phenogroups), and the serological specificities (or blood factors). The tendency in the past to equate agglutinogen with blood factor has been a pitfall, which has given rise to fallacies and errors in studies on the human A-B-O groups and especially the Rh-Hr types. The requirements for a suitable terminology for the A-B-O groups are demonstrated, and it is pointed out that the same principles have universal applicability not only to other blood group systems in man, but also to studies on the blood groups of infra-human species, and antigens of soluble proteins, bacteria and viruses.

THE INVENTION of appropriate terminology and notations is as vital for the progress of the biological sciences as is the use of suitable signs and symbols in the mathematical and physical sciences, and as is lexicography for writers, speakers, and common men who must communicate with one another. Every new branch of science must necessarily develop its own special terminology, in order to facilitate communication and resulting advances in knowledge. Only recently, for example, a special conference was held by workers concerned with the nomenclature of hemoglobin variants, and another dealing with notations for the human chromosomes. This is also an important problem for investigators in the field of blood grouping.

The purpose of a nomenclature is to express facts which must be stated repeatedly in terse symbols or abbreviations. Thus, physicians and nurses use TPR for temperature, pulse and respiration, mathematicians use + and − symbols instead of the words *add* and *subtract*, and letters such as *x* and *y* instead of the phrase "unknown quantity"; and, similarly, blood group workers require their own specialized symbols. Appropriate symbols make possible the terse presentation of information without distortion of facts and without ambiguity. For example, in human blood grouping, "group A blood" is a terse way of saying "blood which has erythrocytes capable of being agglutinated by a serum with the specificity designated anti-**A**, and having in the serum (or plasma) isoantibodies of specificity anti-**B**, if the individual had passed the neonatal period." Since my own interests have principally been in the field of human blood groups, this and almost all other examples I shall cite deal with man. In fact, we are now in the midst of a controversy regarding nomenclature that threatens to interfere with the future progress of our knowledge regarding the Rh-Hr types. This is not the first controversy that has arisen regarding nomenclature for human blood groups. The earlier controversy regarding the A-B-O groups has been resolved reasonably satisfactorily, but, sadly, workers have apparently learned little from the experience, or the present controversy would have been avoided.

* Received for publication May 15, 1961; accepted June 14, 1961.

Nomenclature of the A-B-O Blood Groups

In the case of the A-B-O groups, at one time there were three competing nomenclatures. The four classic blood groups, which are now designated as O, A, B, and AB, were formerly also designated I, II, III, and IV according to Jansky's terminology, or as IV, II, III, and I according to Moss. To the clinician the numbers were appealing—all he had to know was that there are four blood groups, and if his patient needed a transfusion, the blood required could be ordered by number. On the other hand, intelligent use of the lettered designations necessitated at least a rudimentary knowledge and understanding of what the letters represented. The average man is as lazy as he dare be, and if he can get by without bothering to learn something new, he will. Therefore, as long as it was respectable to use the Moss and Jansky symbols, and editors of journals permitted their use, they survived. Unfortunately, there are 4! (or 24) ways of arranging four things, and, in fact, Moss' and Jansky's I and IV were interchanged. This led to misunderstanding, and even to a number of deaths from transfusing blood of the wrong group. This, nevertheless, was not sufficient to stop the Moss and Jansky enthusiasts.[3]

Two things helped to resolve the situation, namely, the exigencies of World War II, and the progress of knowledge regarding the A-B-O groups. The exigencies of war could brook no nonsense, so that abruptly the numbers of Moss and Jansky were dropped. Also, interest in the heredity of the blood groups and their medicolegal application in cases of disputed parentage made individuals aware of Bernstein's triple allele theory, involving the three genes *A*, *B*, and *O*, to explain the heredity of the groups. The mating of parents O $\times$ AB, yielding 50 per cent group A and 50 per cent group B children, is readily understood, but not when written as mating I $\times$ IV, yielding children of groups II and III. Moreover, further studies on serology disclosed at least two varieties of agglutinogen A, resulting in six groups instead of only four, namely, O, A_1, A_2, B, A_1B, and A_2B. As an exercise, one might try to prepare a table of the serology and genetics of the A-B-O groups and subgroups within the narrow confines of the numbers 1, 2, 3, 4, with, if desired, 5 and 6 added. Such an exercise will make clear why, after 1940, in medical and other scientific journals the International A-B-O Nomenclature was used exclusively.

The experience with A-B-O nomenclature teaches a number of lessons. First of all, in science, if semantic difficulties and other problems of communication are to be avoided, a single uniform terminology is essential. The use of more than a single symbol for the same thing necessitates translation from one to the other, and in the process errors can occur, as demonstrated by the experience with the Moss and Jansky numbers. Secondly, no symbol can be completely self-explanatory, except, perhaps, to one already thoroughly familiar with the subject. Group A is so designated because the cells react with anti-**A** serum, but not with anti-**B** serum, etc., and when one learns that the letter O means the failure to react with anti-**A** or anti-**B**, the symbols are to a certain extent self-explanatory. But the serologist also knows that group O means the presence of anti-**A** and anti-**B** in the serum, a fact that the neophyte must learn. To make the notations "clearer" some workers have used the symbols O$\alpha\beta$, Aβ, Bα, and ABo, to represent the four groups. However, symbols are devised for the convenience of workers in a field, and not merely to be intelligible to beginners. It is up to the neophyte to learn the meaning and proper use of the symbols peculiar to the science in which he is interested, rather than for scientists and technical journals to bring their writings down to the level of persons who do not know the

TABLE 1. *The A-B-O Groups and Subgroups*

Group	Reactions of cells with Anti-**A**	Anti-**B**	Anti-$\mathbf{A_1}$	Corresponding genotypes
O	−	−	−	*OO*
$\mathbf{A_1}$	+	−	+	A^1A^1, A^1O, *and* A^1A^2
$\mathbf{A_2}$	+	−	−	A^2A^2 and A^2O
B	−	+	−	*BB* and *BO*
$\mathbf{A_1B}$	+	+	+	A^1B
$\mathbf{A_2B}$	+	+	−	A^2B

subject. Moreover, the attempt to include everything that is known about the subject in the symbols or names would entail changes in terminology every time some new discovery is made, and if certain reported observations later prove to be incorrect, disastrous confusion could result. It is clear, therefore, why symbols like $O\alpha\beta$, $A\beta$, $B\alpha$, and AB*o* have not taken the place of the simpler symbols currently in use.

Another significant fact is that the symbols O, A, B, and AB differ but little from the symbols C, A, B, and AB originally used by Karl Landsteiner,[5] who discovered the blood groups. This is as it should be, because the invention of symbols is the prerogative of the discoverer, who with his insight is also best equipped to find the appropriate symbols to represent the facts he has uncovered.

Some Limitations of the International Nomenclature for the A-B-O Groups

The presently used symbols for the A-B-O blood groups, though vastly superior to the Moss-Jansky numbers, still leave something to be desired. In addition to the meanings assigned to symbols when they are originally defined, there are other interpretations that the symbols themselves may suggest. These additional meanings implicit in the symbols, if incorrect, are dangerous and can do great damage. They insidiously cause one to make certain tacit assumptions, which can give rise to misconceptions that color one's judgment when interpreting findings. Thus, the fact that group A cells react with anti-**A** serum leads one to postulate the existence of an antigenic substance (or agglutinogen) A; similarly, group B cells are considered to have the agglutinogen B, and group AB cells the two agglutinogens A and B. When a similar interpretation is found to hold for other blood group systems like the three M-N types, M, N, and MN, this leads to the tacit assumption of a one-to-one correspondence between agglutinogen and antibodies. Furthermore, since subgroup A_1 cells also react with anti-$\mathbf{A_1}$ serum, while subgroup A_2 cells do not, it may seem natural at first to assume that subgroup A_1 cells have two agglutinogens A_1 and A. In fact, in his book Dacie[1] writes "group A_1 is probably of genotype AA_1, and group A_2 of genotype AO." However, if this interpretation were correct, then in the mating $A_1 \times O$, half the children should be of genotype A^1O and half of genotype AO.

However, the actual situation is quite different.[16, 19] It turns out that three kinds of families with parents $A_1 \times O$ exist; in some all the children are subgroup A_1; in others half are subgroup A_1 and half group O; and there are also occasional families with children half subgroup A_1 and half subgroup A_2. Thus, to account for the subgroups, we can postulate four allelic genes, A^1, A^2, B and O with A^1 and A^2 taking the place of Bernstein's gene A, so that individuals of subgroup A_1 can belong to any of three genotypes, A^1A^1, A^1O, or A^1A^2 (see

Table 1). Gene A^1 appears to give rise to a gene product (or agglutinogen) A_1, with two serological specificities **A**[1] and **A**. Such serological specificities are known also as blood factors. In the case of agglutinogen A_1 the two blood factors are inherited together as a block, so that in the mating $A_1 \times O$, the children either have both blood factors or neither. At any rate, it is already clear that a one-to-one correspondence between agglutinogens and antibodies does not obtain, in the case of the subgroups of A. Instead, it is now well established that every antigenic substance or agglutinogen has multiple corresponding antibodies.[23, 24]

Blood Factor C: Another example of the fallacy of the one-to-one concept is provided by studies on individuals of group O. The serum of such individuals reacts with cells of groups A, B, and AB, but not group O, presumably because they contain a mixture of anti-**A** and anti-**B** agglutinins. In fact, as expected, when group O serum is absorbed by group A cells until it no longer reacts with such cells, it continues to react with group B and group AB cells; and, similarly, after absorption by B cells such serum still reacts with A and AB cells. When titrations are carried out, however, it is found that not infrequently absorption with A cells reduces the titer for B cells, and, reversely, absorption with B cells reduces the titer for A cells. Advocates of the naïve one-to-one hypothesis "explain" this simply by postulating a linkage between molecules of anti-**A** and anti-**B** agglutinins, or by postulating antibody molecules with two combining groups, anti-**A** and anti-**B**. When sufficient group A blood is injected into a group O person to raise his anti-**A** titer, the anti-**B** titer is often raised as well, and absorption of the resulting serum with A cells reduces both the anti-**A** and anti-**B** titers.[21] The one-to-one protagonists then "explain" this by asserting that injections of A cells "nonspecifically" stimulate also a rise in anti-**B** titer, and that the newly formed anti-**A** and anti-**B** antibody molecules link together. A simpler and more reasonable explanation is that group A and group B cells share a common serological specificity to which may be assigned the letter **C**, and that group O serum contains in addition to anti-**A** and anti-**B**, the third antibody anti-**C**. This latter interpretation is set out in table 2, and, diagrammatically, in Figure 1.

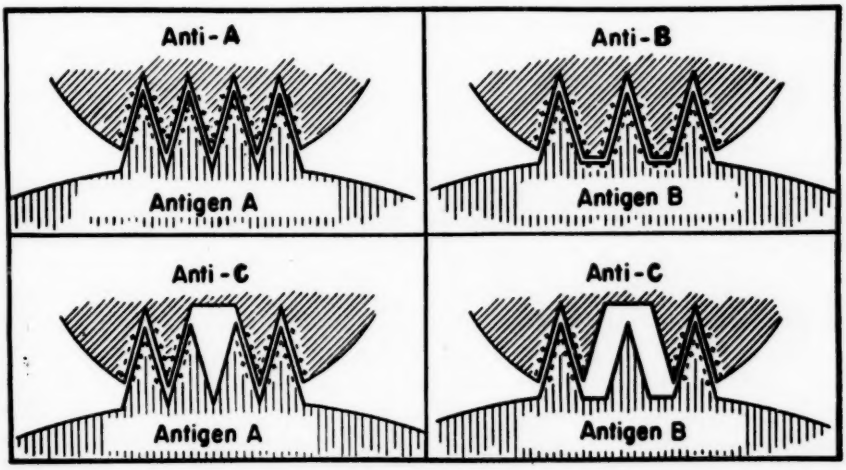

FIG. 1. Diagrammatic representation of the nature of cross-reactions.

TABLE 2. *The Blood Factor C of the A-B-O Groups**

Group	Agglutinogens present	Blood factors	Antibodies in serum
O	—	—	Anti-A, anti-B and anti-C
A	A	A and C	Anti-B
B	B	B and C	Anti-A
AB	A and B	A, B and C	—

* This table shows how the nomenclature of the A-B-O groups is modified to take into account blood factor C.

There is still another phenomenon that refutes the concept of one-to-one correspondence between antigen and antibody, namely, so-called non-reciprocal reactions. For example, anti-rhesus serums produced by immunizing rabbits or guinea-pigs with M. rhesus blood clump preferentially human Rh-positive cells, yet anti-Rh serums produced by injecting Rh-positive blood into human Rh-negative subjects fail to clump rhesus red blood cells. A diagrammatic representation of such reactions is given in Figure 2.

The interpretation of the reactions as suggested by the present author, provides the key to the solution of a medical mystery. There is a hemolytic anemia which occurs in the newborn, and which has been traced to an incompatibility between the A-B-O groups of mother and child, due to the action of the mother's isoantibodies on the baby's red cells. It turns out that in almost all cases the mother is group O and the baby group A or group B, although, theoretically, one might expect a certain number of cases with mother group A and baby group B or AB, and other cases with mother group B and baby A or AB. The explanation of this phenomenon proves to be that the antibody responsible in most cases is not anti-A or anti-B, but anti-C, which only group O persons can produce.[22] Incidentally, it is easier to visualize a simple antibody molecule anti-C passing through the

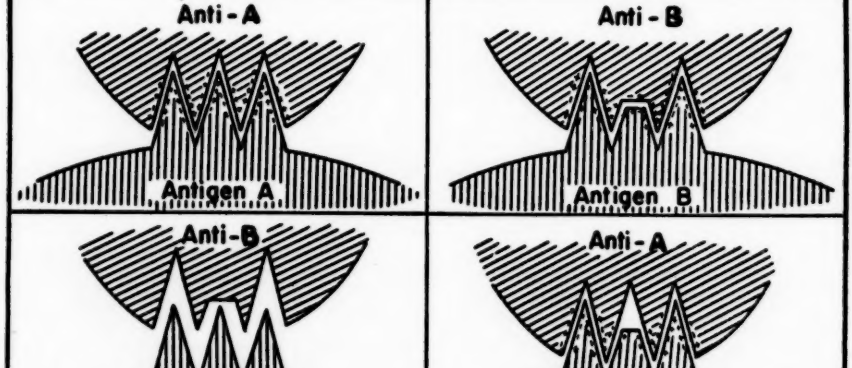

FIG. 2. Diagrammatic representation of the nature of nonreciprocal reactions.

placental barrier, than linked antibodies, anti-A+B.

Here we must pause to see how the terminology of the A-B-O groups can be improved to take these additional facts and principles into account, in order to prevent any false implications by the symbols. An ideal nomenclature must take into account three kinds of biological entities, namely, the genetic determinants or genes, the gene products or agglutinogens, and the serological specificities of each agglutinogen or its blood factors. Therefore, the Committee on Medicolegal Problems of the American Medical Association,[20] in conformity with A. S. Wiener's suggestion, has recommended that the symbols for genes and genotypes be printed in *italics*, symbols for agglutinogens and phenotypes in regular type, and symbols for blood factors and their corresponding antibodies in **boldface** type. For example, gene A^1 gives rise to the agglutinogen A_1, which, as we have seen, has at least three blood factors, $\mathbf{A_1}$, **A** and **C**. In Table 2, the A-B-O nomenclature has been modified to take these principles into account.

Multiple Serological Specificities (Blood Factors) of Agglutinogens

These facts now suggest a new problem. The serological specificities or blood factors of an agglutinogen presumably have a chemical or molecular structural basis. Each blood factor implies a combining site where the antibody can combine with the antigen. One possibility is that each serological specificity requires a separate molecule, so that an agglutinogen with multiple blood factors, though determined by a single gene, is not one but several substances. Another possibility is that only a single molecule is involved, but that each blood factor requires a separate combining site within the molecule. These two explanations do not differ essentially, and do hold in some cases. For example, as Wiener and others have shown,[17] type Rh_1 cells blocked by coating them with anti-$\mathbf{Rh_0}$ still can react with anti-**rh'**, showing that the combining site for anti-$\mathbf{Rh_0}$ is separate from that for anti-**rh'** within the agglutinogen Rh_1. If this explanation held universally, it is obvious that there would have to be a finite limit to the number of blood factors an agglutinogen could have. However, there is, as Wiener[17] has pointed out, a third possibility, namely, that the combining sites for different antibodies may overlap (cf. Fig. 1, 2). For example, Richardson-Jones *et al.*[12] have carried out "blocking" tests which indicate that in agglutinogen A the combining sites determining blood factors **A** and **C** overlap, and, similarly, in agglutinogen B, the combining sites for anti-**B** and anti-**C** overlap. Since combining sites can overlap, it follows that, theoretically, the number of blood factors characterizing any agglutinogen is actually unlimited. In fact, Wiener[19] has asserted repeatedly that the number found is limited only by one's enterprise and ingenuity in searching for and finding antibodies of new specificities. Evidence in support of this prediction has been provided especially by studies on the M-N-S and Rh-Hr types in man, as well as by the studies of Stormont, Owen, Irwin, Briles, and numerous other workers on the blood groups of animals, worked out in greatest detail for cattle.[14] However, in this paper the balance of the discussion will be limited chiefly to the A-B-O blood groups.

Blood Factor H: Using absorbed bovine sera, Schiff[11] first showed the existence of an antibody, anti-**O**, reactive for human group O cells. Landsteiner and Levine[7] found a similar antibody in the serum of certain individuals of subgroups A_1 and A_1B, and since the antibody also reacted with A_2 cells (but not A_1), they also used the designation anti-$\mathbf{A_2}$ for this agglutinin. (Today, the antibody is known simply as anti-**H**, although the reasons that originally led to the introduction of the term anti-**H** are probably fallacious, *i.e.*, to distinguish two

TABLE 3. *Reactions of Cells of Subgroups A_1 and A_2 with Various Antisera**

Blood of subgroup	Reactions with Anti-A	Anti-A_1	Anti-O (or anti-A_2)	Blood factors present
A_1	+	+	−	A and A_1
A_2	+	−	+	A and O (or A_2)

* This table illustrates the distinction between antigens and their serological specificities as applied to the subgroups of A.

forms of the antibody, a "natural" form, designated anti-**H**, and an "immune" form, called anti-**O**.) According to Landsteiner and Levine's observations, the reactions of cells of the subgroups A_1 and A_2 are as shown in Table 3; thus, blood of subgroup A_1 has the two factors **A** and $\mathbf{A_1}$, while blood of subgroup A_2 has the two factors **A** and $\mathbf{A_2}$. For a short time Landsteiner and Levine therefore used the terminology AA_1, AA_2, AA_1B and AA_2B for the four subgroups. When it was realized from genetic studies that factors **A** and $\mathbf{A_1}$ are merely two serological specificities of a single antigenic substance A_1, and, similarly, for **A** and $\mathbf{A_2}$, the symbols for the two subgroups were then shortened to A_1 and A_2 respectively.

Other Blood Factors of the Group A and Group B Cells: Antisera of specificities anti-**A** and anti-**B** are usually produced by immunizing human volunteers with solutions of group substances, and they have also been produced by immunizing rabbits with human red cells,[16] and still others have been prepared from seed extracts (lectins).[11] No matter what the source of the reagent may be, when human red cells are typed by two or more potent reagents in parallel, identical reactions are obtained, which would appear to indicate that all the reagents contain antibody molecules of identical serological specificities. On the contrary, there is evidence that anti-**A** and anti-**B** sera, whether of "natural" or "immune" origin contain a spectrum of antibodies of different though related specificities. This has been demonstrated by studying the cross reactions of such reagents with red cells from infrahuman species.

Schiff and Adelsberger[12] found that when selected rabbits are immunized with human group A blood, antisera are often obtained which are hemolytic for sheep cells in high dilutions, while antisera prepared by injecting sheep cells into rabbits may have high titers for human group A, but not for group B cells. This indicates that human group A red cells share a serological specificity with sheep cells, and the common blood factor has been designated $\mathbf{F_A}$, because of its similarity to the Forssman antigen. Further studies on the cross reactions of anti-**A** serum with the blood cells of infrahuman species have disclosed still other blood factors, *e.g.*, a blood factor shared with pig red cells and therefore designated $\mathbf{A^P}$ has been described in the recent literature[26] on the subject.

Similarly, studies of the cross reactions of "natural" and "immune" anti-**B** serum have disclosed that these may possess a multiplicity of serological specificities. For example, group B cells share a blood factor with rabbit cells, as shown by the fact that some anti-**B** sera are absorbed almost as well by rabbit cells as by human B cells, while other anti-**B** sera react exclusively with human B cells. Studies on the blood cells of other animals, such as guinea-pigs, rats and opossums, have disclosed still further serological specificities, so that human group B cells may be assigned a

series of blood factors designated $\mathbf{B}_{\mathrm{I}}$, $\mathbf{B}_{\mathrm{II}}$, $\mathbf{B}_{\mathrm{III}}$, $\mathbf{B}_{\mathrm{IV}}$, etc., depending on the nature of the cross reactions of the corresponding antisera.[2, 9]

Nature of Agglutinogens

Although the concept of an agglutinogen as the gene product is convenient and useful one must bear in mind that agglutinogens have never been extracted in pure form from red cells, and the existence of such entities is inferred rather than proved. If, in conformity with the gene theory, we postulate the existence at a certain locus on a pair of chromosomes of genetic determiners or functional genes for the A-B-O groups, then every individual has a pair of genes of each variety. Thus, every person's A-B-O genotype comprises a pair of genes, one inherited from each parent. Accordingly, appropriate *in vitro* tests will establish an individual's phenotype, which represents the product of a *pair* of genes, and there is no direct way to determine the effect of a single gene. The available evidence argues against the view once held that the agglutinogen is the *direct* product of its corresponding gene; besides tests on spermatozoa (or ova) each of which theoretically should contain but a single A-B-O gene yield no useful results. Therefore, the product of the hypothetical A-B-O genes must be deduced from the phenotypic effects of pairs of genes comprising the individual's genotype. This depends on the tacit assumption that the products of the genotype is the simple sum of the products of its pair of genes.

As a matter of fact, there is evidence that the phenotype is not merely the sum of the products of the two genes that make up the genotype. For example, red cells of individuals of subgroup A_2 (genotype A^2O) react much more strongly with anti-**A** sera than cells of subgroup A_2B (genotype A^2B), although both presumably depend on the same single gene A^2. The answer may be that the two genes of the genotypes combine to produce not two (or more) agglutinogens, but only one with two determinant groups. In A_2B cells there may be steric interference within the agglutinogen molecule, and the propinquity of the side chain for B may interfere with the union of anti-**A** with the side chain for A. Whatever the correct explanation, the concept of a gene and its product the agglutinogen is a useful approximation that facilitates one's understanding of the hereditary transmission of the blood groups.

When the blood of individuals has been found to possess several serological specificities or blood factors it is important to know whether these represent a single agglutinogen or more than one agglutinogen. This is determined by family studies. For example, in the mating AB $\times$ O, the factors **A** and **B** separate in the children proving that they are attributes of different agglutinogens. On the other hand, in the mating $A_1 \times O$, with A_1 and O children, the children either have all the factors **A**, $\mathbf{A}_1$, **C**, $\mathbf{F}_{\mathrm{A}}$, or none, showing that **A**, $\mathbf{A}_1$, **C** and $\mathbf{F}_{\mathrm{A}}$ are attributes of a single agglutinogen. This application of genetics to serology is a most fruitful development, and this alone justifies the crytallization of Immunogenetics as a separate scientific discipline.

Terminology

An appropriate terminology for the A-B-O groups should therefore make possible two tabulations. One table should be a list of observed phenotypes, giving the known serological specificities of the red cells and the antibodies in the plasma. While the symbols for the phenotypes should be terse—in the nature of mnemonics—and permanently fixed, the listing of blood factors should be detailed and subject to modification as new observations are made that add new specificities and modify or correct previously reported observations. Such a partial listing is shown in Table 4. Another useful table would be a list of the postulated blood group genes

TABLE 4. *Present Status of the A-B-O Groups (Modified International Nomenclature)*

Phenotype	Red cells (blood factors)*	Plasma (isoantibodies)
O	H	Anti-A, anti-A_1, anti-B and anti-C
A_1	A, A_1, F_A, C	Anti-B (and anti-H)†
A_2	A, H, F_A, C	Anti-B (and anti-A_1)
B	B_I, B_{II}, B_{III}, B_{IV}, C	Anti-A and anti-A_1
A_1B	A, A_1, F_A, C, B_I, B_{II}, B_{III}, B_{IV}	(Anti-H)
A_2B	A, H, F_A, C, B_I, B_{II}, B_{III}, B_{IV}	(Anti-A_1)

* The list only includes those blood factors the existence of which has been clearly established up to the present time. This list is subject, of course, to modification by addition.

† Antibodies listed in parenthesis are not present regularly but only in a small proportion of individuals.

and the serological specificities of their postulated gene products or agglutinogens. Such a partial listing is shown in table 5.

The lists in Table 4 and Table 5 were deliberately made incomplete, because only those phenotypes and genes are listed concerning which our knowledge is reasonably complete. For example, Table 4 might also have included the rare phenotypes A_3, A_3B, A_o and O_h, and others, while Table 5 might also have listed gene I^{A_3} and others. However, such listings should perhaps be deferred until the facts concerning these rarer phenotypes and genes have become clearer.

The principles of serology, genetics and nomenclature described here have universal applicability. They have been applied by the author with signal success to the complex Rh-Hr system in man, as well as to other blood group systems, notably, the M-N-S system. The same principles are applicable to the infrahuman species. In fact, the principles have applicability to a great variety of immunological problems, including even soluble antigenic substances and antigens of bacteria and viruses.

Blood Factors as Extrinsic Attributes of Agglutinogens

In 1952, the author was requested to write a review on the Rh-Hr blood types for publication in Bacteriological Reviews. With I. B. Wexler[23] he then prepared a review on "The Mosaic Structure of Red Blood Cell Agglutinogens," in which was pointed out the multiplicity of blood factors which characterize each antigenic substance or agglutinogen. This review was returned by the editor with the comment that a referee had criticized it adversely on the ground that he and other investigators considered that there is no essential difference between agglutinogens and blood factors; in fact, most workers used the two terms interchangeably. We responded that the very purpose of the review was to correct this misconception, whereby an object (the agglutinogen) is confused with its serologic attributes (the blood factors) —for as Shakespeare so aptly stated, "All is not gold that glitters." The reader may still find it difficult to accept the fact that every agglutinogen—indeed, every combining group within the agglutinogen molecule —theoretically can have an *infinite* number of serological specificities or blood factors. The explanation is that blood factors are *extrinsic* attributes of agglutinogens. A few parallel examples will be cited which may perhaps make this concept easier to grasp.

One of the *intrinsic* attributes of an object is its *mass*, or the amount of matter which it contains. With this mass one is accustomed to associate a fixed weight; in fact, a common mistake is to confuse mass and weight, just as most serologists confuse the terms agglutinogen and blood factor. Actually, an object of fixed mass has an

infinite number of possible weights, depending where the weighing is done, at the equator, at one of the polar regions, on a mountain, in a valley, in a moving elevator, etc., and the weight can even be negative, as when an object of low specific gravity is weighed under water. In fact, if one could imagine a race of human beings spending their entire lifetimes on an artificial satellite revolving about the earth, under a state of weightlessness, the meaning of weight might be difficult for them to appreciate, and if such individuals had their own dictionary the word "weight" or its equivalent might well be missing from it. Thus, while the mass of an object is an *intrinsic* attribute, its weight is an *extrinsic* attribute and can assume any of an infinite number of values. Similarly, the serological specificities or blood factors of an agglutinogen are extrinsic attributes, so that there can be an infinite number corresponding to any single combining group (an *intrinsic* attribute of the agglutinogen molecule).

Another example is provided by the protective coating of paint on an automobile. While the chemical composition of the paint is one of its *intrinsic* attributes, the color of the paint is an *extrinsic* attribute. The chemical composition is fixed, but the apparent color will vary with the illumination under which it is observed, and within limits an infinite number of possibilities exist. In fact, if one imagines an earth populated entirely by totally color blind people, there would be no such attribute as color (and the word would be omitted from their dictionaries), just as the existence of blood factors depends on the discovery of appropriate antisera to demonstrate them.

The realization that each agglutinogen can have an infinite number of blood factors should be the impetus to further investigations on the blood groups, by stimulating a search for antisera with new serological specificities. The types of antibodies that may be produced will vary not only with the antigenic substances injected but also with the animal immunized. Thus, so called "natural" anti-**B** and isoimmune anti-**B** agglutinins in human sera, as well as anti-**B** produced in rabbits or in chickens, or anti-**B** extracts from seeds could be expected to show differences in specificity. Similarly, anti-**M** and anti-$\mathbf{Rh_o}$ produced by rabbits in response to injections of rhesus monkey blood would not be expected to be identical with similar antibodies produced by man in response to injections of blood of other human beings. A practical consequence was that this suggested the origin of the "natural" isoagglutinins anti-**A** and anti-**B** of human sera. As pointed out by the author,[18] these antibodies are probably not of "spontaneous" or hereditary origin, but are instead of heterogenetic immune origin, and depend on the ubiquitous presence of A-like and B-like antigens in a great variety of animals, plants and microörganisms. Thus, it is inevitable that every individual will come

TABLE 5. *Partial List of the A-B-O Allelic Genes and Agglutinins*

Genes* a	b	Agglutinogens	Blood factors
I^0	O	O	**H**
I^{A_1}	A^1	A_1	**A, A_1, F_A, C**
I^{A_2}	A^2	A_2	**A, H, F_A, C**
I^B	B	B	**B_I, B_{II}, B_{III}, B_{IV}**, etc., **C**

* The symbols under columns a and b present alternative methods of designating the A-B-O genes.

into contact with such antigens, either in foods, or by infection, or in parasitic microörganisms infecting the gastro-intestinal tract, *e.g.*, some strains of B. Coli have been found to have A-like while others have B-like antigens. In support of this explanation can be cited certain interesting investigations on germ-free chickens of Springer *et al.*[13] While normal chickens have "natural" antibodies of anti-**B** specificity, such antibodies are lacking in chickens that have been reared in a germ-free environment. When such germ-free chickens are allowed to ingest B. Coli of the strains with B-like antigens, they promptly form high titers of anti-**B** agglutinins.

On the other hand, as has been pointed out, the tendency to confuse agglutinogens and blood factors has led to the tacit adoption of the one antigen/one antibody concept. In human blood grouping this has given rise to the C-D-E concept of Rh-Hr serology and nomenclature. Since the numerous fallacies and errors that these concepts have caused have been discussed elsewhere,[19, 25] this will not be repeated here. Instead, a new kind of fallacy invoked by Vogel, Pettenkofer and Helmbold[15] will be described. According to Vogel *et al.*:[15] "Pasteurella pestis possesses an antigen which by means of experiments with rabbits has been shown to be very similar to the H-antigen (of the ABO system). This may justify the conclusion that patients of blood group O, who on account of their own antigen H are unable to produce any anti-H antibody, have had a very unfavorable prognosis in the case of an infection with plague. Really, the gene O is very frequent in places where few or no epidemics of this kind have occurred, and, vice versa, it is rare in the known plague centers. The virus causing variolla (smallpox) has been shown by experiments to possess an antigen which is at least very similar to the A antigen of the ABO system. It seems highly probable that the humoral resistance against smallpox is more effective in patients of blood group B and O, who carry an anti-A in their serum, than in patients without this antibody (blood group A or AB). The geographical distribution of the A gene in the old world supports the hypothesis of a selective disadvantage of the carriers of this gene when infected with smallpox. This implies that in areas with smallpox as well as plague the gene B would increase due to selection against A and O." Vogel *et al.* cite in support of their hypothesis also the paper of Livingstone[8] who asserts that among West African Negroes those with the worst pock marks show an increased frequency of blood group A.

This superficially attractive hypothesis of Vogel *et al.*, depends again on the fallacious assumption of one-to-one correspondence between antigen and antibody. It is erroneously assumed that group A persons cannot become adequately immunized to smallpox because of the presence in the virus of the antigen A. The fallacy here is that it is hardly likely that the A antigens of smallpox virus and human group A individuals are identical, and, in addition, smallpox virus must have an unlimited number of other serological specificities that group A persons lack. Thus, being group A is hardly likely to prove a special disadvantage in the face of a smallpox infection, and, similarly, being group O would hardly be a handicap in the event of a plague epidemic. At any rate, the hypothesis regarding smallpox can be put to a simple blind test. Firstly, one can record the results of primary smallpox vaccinations in a series of babies, or of secondary vaccinations in older children, or in adults. *After* this has been done, the blood group of each individual can be determined and the presence of any association between ABO group and the results of vaccination determined. The result of this simple experiment should help to confirm or refute the hypothesis of Vogel *et al.* It is most important, however, that the experiment

be carried out blind in the order described, because prior knowledge of a subject's group could subconsciously bias one's readings of the results of vaccination.

In blood grouping investigations, one is dealing with antigenic substances of unknown chemical structure (except, perhaps, for the A-B-O substances) and antibody molecules (presumably gamma globulin) whose combining groups are inaccessible to chemical analysis. To determine the chemical nature of the antigenic substances therefore, they must first be extracted in pure form and then analyzed. Landsteiner and Harte[6] did extract the A-B-O group substances from saliva, controlling the degree of purification by tests for serological activity, and have subjected such preparations to chemical analysis. Incidental to such studies, they found that upon partial denaturation, preparations of A substance lost their capacity to inhibit anti-**A** isoagglutinins, but retained their ability to inhibit, in high dilutions, the lytic activity of rabbit immune anti-**A** for sheep cells. This indicates that the factors **A** and $\mathbf{F_A}$ probably depend on different structures within the antigen molecule.

Inhibition tests with simple sugars have been carried out in an attempt to deduce the chemical structure of A-B-O and also Rh-Hr substances. While such studies may provide a clue, one must bear in mind that the tests are necessarily restricted to only a few antisera among the theoretically unlimited number of serological specificities which might be tried. Therefore, attempts to draw sweeping conclusions from such limited investigations are certainly dangerous and premature, unless supported by chemical analytical data on the antigens themselves.

Nature of Antibody Specificity

One of the most important attributes of antibodies is their specificity. From the foregoing discussion, however, it should be clear that no or hardly any antibody is absolutely specific for the antigen which elicited its production, because, for example, antibodies reactive for sheep cells can result from such diverse stimuli as injections into rabbits of human group A cells or of guinea-pig kidney, or infection of human beings with the virus of infectious mononucleosis. Thus, antibodies, besides being specific also cross-react, and expressions such as "cross-reacting antibodies" are redundant.

It is clear, therefore, that the generally held concept of the high degree of specificity of antibodies is incorrect; in fact, this is merely another aspect of the false one antigen-one antibody idea. Yet it is possible to identify agglutinogens specifically by serological means—not by a single antibody, but by the specific *set* of serological specificities or blood factors that characterize it. One of the chief obstacles to the general acceptance of this fact is that in one's everyday work in blood banks, technicians get along perfectly well with by a *single* reagent of each specificity, *e.g.*, only one anti-**A** and one anti-**B** serum. The reason for their success is that they have available further information, namely, that the blood is from a human being, and in man every group A blood has *all* the factors **A**, $\mathbf{F_A}$, and $\mathbf{A^P}$, and every group B blood all the factors $\mathbf{B_I}$, $\mathbf{B_{II}}$, $\mathbf{B_{III}}$, and $\mathbf{B_{IV}}$. That is why any pair of anti-**A** and anti-**B** sera will give the same correct

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In this connection, the following incident which occurred to the speaker may be of interest. At the Office of the Chief Medical Examiner of New York City, blood grouping tests are carried out routinely in all cases of violent death, and suspected homicide, so that if later blood stains are found, the blood group of the stain can be compared with that of the deceased. In one such case, routine grouping tests on the postmortem blood showed it to be group A; however, the red cells failed to clump in anti-**M** or anti-**N**, while the serum contained agglutinins for group O cells. Closer inspection showed that the red cells were smaller than usual for human blood, and precipitin tests were positive for sheep blood. Investigation then disclosed that the messenger, through error, had picked up the bottle of sheep blood from the Wassermann laboratory instead of the blood of the deceased.

answer, so long as these tests are not extended to blood from infrahuman species.

For the same reasons, the conclusion that urine contains glucose because it gives a positive Benedict's test will usually be correct, though occasionally the presence of lactose or pentose or an entirely different reducing substance may trip up the technician. And it is easy to understand why not so long ago people who spent their entire lives in the place they were born insisted that the world was flat, and found the fact of its roundness inconceivable. Nowadays everyone is taught the world is round, even those who never intend to travel. By the same token, every technician should be taught the difference between agglutinogens and blood factors, even though they do not intend to do advanced serological work.

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The Use of Papain and Bromelin in Blood Bank Screening Procedures:*

An Evaluation and Comparison with Standard Technics

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A comparison of l-cysteine activated papain and bromelin with saline, albumin and anti-human globulin (AHG) technics in the detection of antibodies to red cells indicated that the enzyme technics are more sensitive than saline and albumin technics; are not significantly different from the AHG technic in the detection of anti-D, anti-C and anti-e; are more sensitive in the detection of anti-E, anti-c and immune anti-A; are less sensitive in the detection of anti-Kell, anti-Duffy, anti-M and anti-S—the corresponding antigens of which they alter to prevent a positive AHG reaction. Serum inhibition of papain was prevented by the use of 1% papain up to a dilution of 1:32; enhancement of titers resulted from the use of 0.1% enzyme thereafter. A 15-minute incubation period was found to be optimal. Bromelin solutions were found to be much more stable than papain. It was concluded that although these enzyme technics are simple, economical and satisfactory within the limits applying to any enzyme technic, they are valuable adjuncts only and cannot replace the AHG test in prenatal and pretransfusion antibody screening.

* Received for publication February 7, 1961; accepted April 14, 1961.

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IN NO AREA of the Clinical Laboratory do the personnel carry so great a responsibility for the life and welfare of the patient as in the Blood Bank Laboratory. Naturally-occurring antibodies and previous pregnancies introduce the possibility of incompatibility even into the first transfusion. Multiple antibodies may be produced in response to a single transfusion. Multiple transfusions, so common in this day of extensive surgical procedures and improved methods of treating debilitating diseases, are even more prone to give rise to antibodies that may render future transfusions and pregnancies hazardous. For these reasons it is incumbent on Blood Bank personnel to use the best technics available and, in particular, to evaluate any new procedure very critically before adopting it.

The anti-human globulin (AHG) technic can and does detect most clinically-significant antibodies when it is properly performed with adequately prepared and

standardized serum. However, the time-consuming and meticulous technic demanded tends to discourage its use by the technologist who may be already overburdened or is high-pressured by the attending physician to provide blood for his patient within minutes of the time that it is requested. The early enzyme technics, which involved pretreatment and repeated washings of cells followed by a reacting period with the serum to be tested, were as cumbersome and time-consuming as the AHG technic. Using l-cysteine-activated papain, Loew[10] developed a simultaneous enzymation and sensitization test calling for a two-hour incubation period. Stern and his associates,[12] using activated papain, reduced the incubation period to 30 minutes. The instability of activated papain at 4 C. and the inconvenience of having to use it within a few minutes of the time it was thawed after being stored at −20 C. led to a more extensive study of bromelin. Gray[5] reported increased anti-A, anti-B, and anti-Kell activity with bromelin but at the same time observed a limitation (also reported for trypsin, ficin, and papain) which is probably inherent in enzyme action. This is the alteration of certain antigens, notably M, N, and Duffy, since these factors cannot be detected by the AHG technic after enzyme treatment. Pirofsky,[11] however, reported a one-stage bromelin technic by which he detected M, N, and some Duffy antibodies. He also concluded that the 15-minute bromelin test was faster, more sensitive, and less likely to give false positive results than the cysteine-activated papain technic. He claimed that it equalled or even surpassed the AHG technic in sensitivity, reproducibility, and specificity but did not require the extended time and meticulous technic of the latter.

The study herein reported was carried out in an attempt to decide whether these papain and bromelin technics are as specific and sensitive as their proponents claim and whether they could or should replace any of the standard technics (particularly the AHG technic).

Materials and Methods

Sera from Rh-negative patients of the Health Center Prenatal Clinic, from patients presenting compatibility problems, and from healthy Blood Bank donors (as controls), as well as commercial typing sera were tested. Three AHG sera—A and B commercially produced in rabbits and C commercially produced in rabbits and goats—were used for the Coombs tests. A pool of Group O cells of known antigenic structure in an approximately 5 per cent suspension in Alsever's solution was used for the screening tests. Eight Group O cells of known antigenic structure were used, as directed in the instructions accompanying each lot, for the identification of antibodies. Selected cells of known antigenic structure were used for the titers. The 0.5 per cent bromelin solution at *p*H 5.5 was prepared by suspending 0.5 g of "Bromelain" (No. 179, Hawaiian Pineapple Co., Ltd.) in 100 ml. of buffer solution composed of 88 parts of 0.15M KH_2PO_4 and 12 parts of 0.15M Na_2HPO_4 and filtering the suspension by suction through Whatman No. 1 filter paper. The filtrate was dispensed into dropper bottles or vacutainers in approximately 2 ml. amounts. Some was stored at 4 C.; the rest, frozen and stored at −20 C. Bromelin with preservatives was prepared by adding 100 mg. sodium azide (Fisher Scientific Co.) and 50 mg. "Actidione" (Upjohn Co., cycloheximide) per 100 ml. of filtrate before dispensing into tubes. The 0.05 per cent solution was prepared by making a 1:10 dilution of the 0.5 per cent solution with isotonic saline just prior to use. The 1 per cent papain solution was prepared according to the method of Stern.[12] The 0.1 per cent papain was prepared by diluting the 1 per cent solution 1:10 with isotonic saline.

TABLE 1. *Comparison of Results Using 1 Per Cent and 0.1 Per Cent Papain with Anti-D Serum and O, Rh-Positive Cells*

Sera	Papain	Dilutions 1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
1	1.0%	++++s	++++s	+++	+++	++	+	−	−	−
	0.1%	−	−	−	−	−	±	+	−	−
2	1.0%	++++	++++	++++	++++	+++	+++	++	+	−
	0.1%	−	−	−	−	±	+	++	+	−
3	1.0%	++++s	++++	+++	++	+	+	−	−	−
	0.1%	−	−	−	+	+++	+++	++	+	−

The enzyme screening tests were performed by thoroughly mixing two drops of the test serum, one drop of the pooled cells, and one drop of enzyme in a 10×75 mm. tube. Enzyme stored at −20 C. was thawed at 37 C. or lower and, in the case of papain, used within ten minutes. After centrifugation for 30 seconds at 2,000 rpm in a Clay-Adams serofuge the tubes were examined for macroscopic agglutination by shaking them gently until the sediment was completely loosened, and positive results were recorded as + (small but plainly visible clumps) to ++++s. The tubes were then incubated for 15 and 30 minutes in a 37 C. water bath, centrifuged, and examined as before. The results were recorded as the "enzyme screen." The saline screens were performed by mixing two drops of the test serum and one drop of the pooled cells in a 10×75 mm. tube, centrifuging, examining, and recording as for the enzyme technic. The tubes were then incubated in a 37 C. water bath for one hour, centrifuged, and examined as before. The results were recorded as the "saline screen." After three washings with fresh saline according to the standard AHG technic, one drop of AHG serum was added. After 30 seconds centrifugation the tubes were again examined for agglutination, and the results were recorded as the "AHG screen." The albumin screens were carried out in the same manner as the saline screens except that cells suspended in 22 per cent bovine albumin were used.

For the titrations, beginning with a minimum of 0.3 ml. of serum, doubling dilutions from 1:2 to 1:4,096 were made. The pipette used for measuring the serum was discarded and a single clean pipette was used for making and dispensing the dilutions. For the albumin titers both the dilutions and the cell suspensions were made with 22 per cent bovine albumin. For the saline, AHG, and enzyme titers both the dilutions and the cell suspensions were made with isotonic saline. 0.1 ml. of a 4 per cent suspension of red cells having the antigen for which the serum contained the specific antibody was added to each tube. For the enzyme titers 0.05 ml. (one drop) of the enzyme being tested was added to each tube. After thorough mixing the tubes were centrifuged for 30 seconds and examined for agglutination. A + reaction was taken as the end point. Following this the enzyme titers were incubated for 15 and 30 minutes and examined for agglutination after a 30 second centrifugation. The results were recorded as the "enzyme titers." The saline and albumin titers were incubated for one hour in a 37 C. water bath, centrifuged, and examined. The results were recorded as the "saline" and "albumin" titers respectively. The saline titers were then converted to AHG titers by washing three times with fresh isotonic saline, adding a drop of AHG serum, and centrifuging for 30 seconds. The results were recorded as the "AHG titer."

TABLE 2. *Stability Testing of Bromelin Solutions*

Solution	Storage conditions	Results
Bromelin without preservatives	4 C.	Solution darkened after two weeks. Sensitivity maintained until discarded because of fungal contamination at four weeks.
	—20 C.	Sensitivity maintained during the four-month testing period.
Bromelin with "Actidione" and sodium azide	4 C.	Solution slightly darkened. Sensitivity maintained during the four-month testing period.
	—20 C.	Sensitivity maintained during the four-month testing period.
	—20 C. followed by thawing and storage at 4 C.	Sensitivity maintained until the end of the testing period (two and one-half months after thawing).

The absorption studies were carried out as follows: The serum to be absorbed was diluted with an equal amount of isotonic saline. A portion was set aside for the preabsorption titers. To the other portion was added an equal amount of thoroughly washed and packed cells of the desired specificity. After incubation in a 37 C. water bath for one hour and at 4 C. overnight, the tubes were centrifuged to pack the cells completely. The serum was removed and tested as in the AHG screen. If antibodies were still demonstrable, the absorption was repeated with fresh packed cells until the AHG screen was negative. The enzyme screening tests were then performed on the absorbed serum. If they were positive, doubling dilutions were made, and enzyme titers were performed. As a control, AHG and enzyme titers were performed on the unabsorbed serum.

Results

AHG serum B was selected for routine use because, when used with cells sensitized by low-titer anti-D serum, it gave stronger agglutinates that were more readily loosened from the bottom of the tube; it was less expensive and more readily available than AHG serum C; and it did not appear significantly less sensitive under the conditions of testing. A 30-second centrifugation period was selected because it gave crisper agglutinates.

Prozones occurred with both papain and bromelin when 0.1 per cent enzyme was used beginning with the 1:4 dilution of serum as suggested by Stern.[12] Using 1 per cent and 0.1 per cent papain in duplicate titers with various sera, it was determined that the prozone could be avoided by using the 1 per cent solution with dilutions up to and including 1:32. Using 0.5 per cent and 0.05 per cent bromelin, it was found that the prozone could be avoided by using the 0.5 per cent solution with serum dilutions up to and including 1:16. The use of dilute enzyme with the higher serum dilutions resulted in significantly higher papain titers as shown in Table 1 but did not affect the bromelin titers. The sensitivity of "Bromelase" (Dade Reagents) was comparable to that of the solutions prepared from "Bromelain." Preliminary tests using various concentrations of bromelin at various *p*H's demonstrated the senstivity of the 0.5 per cent solution at 5.5 *p*H.

The results of stability testing of bromelin solutions given in Table 2 indicated that loss of activity under the conditions specified is not significant and is unlikely to affect the usefulness of bromelin for

routine testing. Activated papain retained its reactivity up to six or eight weeks at −20 C.

Identical results were obtained with 92 anti-D sera screened by the AHG, papain, and bromelin technics. Three other anti-D sera were detected by two of the three technics. When fresh specimens were obtained from these patients one month later than the original specimens, they were found to be positive by all three technics. These data are summarized in Table 3. Anti-D antibodies were, with one exception, detected earlier and on lower titers with the AHG and the enzyme technics than with the albumin technic. Fifty-seven sera containing rarer antibodies were tested. Of these, 50 were positive by the AHG technic; 30, by the bromelin; and 28, by the papain —as shown in Table 4. Some negative enzyme tests were converted to positive by subsequently applying the AHG technic. In addition to the sera included in Tables 3 and 4, nine AHG-negative sera were papain-positive; and six, bromelin-positive. This was shown to be due to nonspecific cold agglutinins. In most instances placing the tubes in the water bath for five to ten minutes caused these cold agglutinin reactions to disappear. Three hundred sixty-nine control sera screened by papain, bromelin, and AHG were uniformly negative.

TABLE 3. *Comparison of Sensitivity in Detection of Low-Titer Anti-D in 95 Sera*

Results	No. of sera
*AHG +, Brom. +, Pap. −	2
AHG −, Brom. +, Pap. +	1
AHG +, Brom. +, Pap. +	92

* AHG serum B used

Of 89 AHG and papain titers determined simultaneously, 39 were identical; 21 showed higher papain values; and 29, lower papain values. These results are summarized in Figure 1. Thirty titers comparing papain and AHG with bromelin were performed. The AHG and bromelin titers

TABLE 4. *Detection of Rarer Antibodies by Different Technics*
No. positive/No. sera tested

Antibody	AHG**	Brom.	Brom. + AHG	Papain	Papain + AHG
C	3/3	3/3		3/3	
C^w	1/1	1/1		1/1	
E	3/5	3/5		5/5	
c	5/6	6/6		6/6	
e	3/3	3/3		3/3	
*M	3/3	1/3	0/2	2/3	0/1
S	2/2	1/2	1/1	1/2	0/1
s	1/1	0/1	1/1	0/1	0/1
*P	1/5	5/5		4/5	
V	2/2	2/2		2/2	
K	14/14	2/14	3/3	0/14	3/4
k	3/3	2/3	1/1	1/3	1/1
Fy^a	7/7	1/7	2/5	0/7	3/5
Jk^a	2/2	0/2		0/2	
Total	50/57	30/57	8/13	28/57	8/13

* All tests performed at 20 C.

** AHG serum B used for all tests. AHG serum C used for anti-M, anti-K, and anti-Fy^a with no significant difference in results.

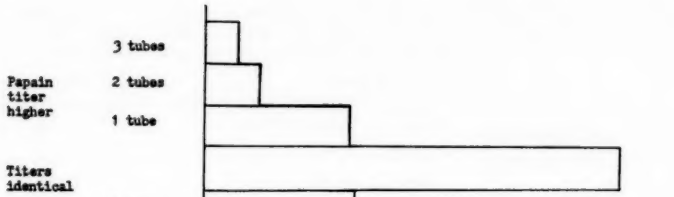

FIG. 1. Comparison of sensitivity of AHG and papain in titration of 89 anti-D antibodies.

were identical in 12 of these; ten gave higher bromelin titers; and eight gave lower bromelin titers. These are reproduced in Figure 2. Figure 3 compares the results of papain and bromelin titers. Fourteen were identical; 11 were higher with bromelin; and five were lower with bromelin. Two sera containing both natural and immune anti-A antibodies were tested to study the sensitivity of the bromelin and papain technics in the detection of antibodies in the ABO system. Saline, AHG, and enzyme titers showed that AHG, papain, and bromelin were equally sensitive but significantly more sensitive than saline in the detection of combined natural and immune antibodies. After neutralization of the natural antibodies by incubating two parts of the serum with one part of Witebsky substance for one hour at room temperature, saline, AHG, and enzyme titers were performed. The sensitivity of both papain and bromelin was found to be significantly greater than that of saline in both instances. With one of the sera it was significantly greater than that of the AHG technic also. These results are shown in Table 5. The experiment devised to study the effects on papain and bromelin titers of the absorption of antibodies detectable by the AHG technic showed that five of the absorbed sera were negative by the enzyme technics; 11 were positive by one or both. The titers before and after absorption are shown in Table 6.

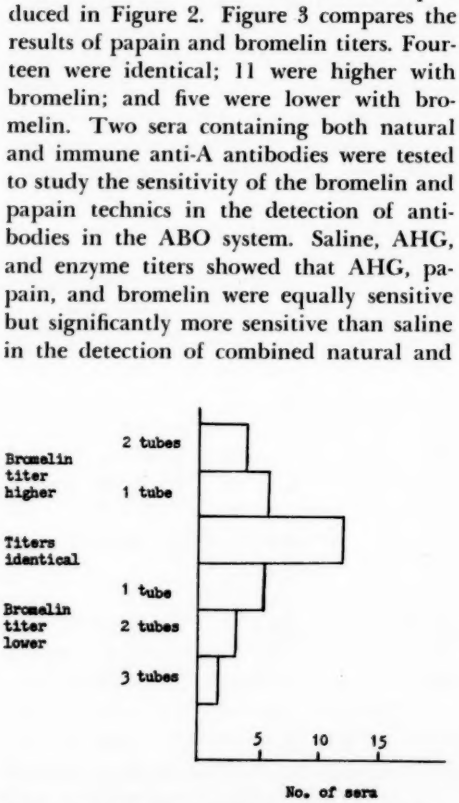

FIG. 2. Comparison of sensitivity of bromelin and AHG in titration of 30 anti-D antibodies.

Discussion

In matters of such consequence as the transfusion of blood and the diagnosis of hemolytic disease of the newborn every possible precaution must be taken to insure the life and welfare of the patient. To do this, the technics used for the detection of antibodies and compatibility testing must be sensitive, specific, and reproducible. Because blood lost by massive, acute hemorrhage must be replaced as rapidly as possible, rapidity without sacrifice of accuracy is an equally desirable

feature. From the viewpoint of the busy technologist ease of preparation and stability of reagents, together with simplicity of performance, are no less desirable. Finally economy in price and quantity of materials used recommend a test to those interested in keeping hospital costs as low as possible without jeopardizing the patient. These are the criteria used in this attempt to evaluate papain and bromelin technics by comparing them with standard technics. In some cases re-evaluation of these standard technics is also indicated.

The economy, simplicity of performance, and ease of reading saline tests are more than offset by the failure of this technic to detect the more clinically significant incomplete antibodies. Serum, plasma, albumin, and plasma-albumin technics detect many of the incomplete antibodies. However, the difficulty of obtaining sufficient serum or plasma lacking antibodies for use as diluting and suspending agents, the expense and prolonged incubation period of albumin, and the difficulty of reading the final results are definite disadvantages. Furthermore, they failed to give a sensitivity approaching that of the AHG, papain, and bromelin technics in this study.

In keeping with the findings of Haberman[7] and other investigators, the AHG technic yielded a very high percentage of true, and no false positives. As a review of Figures 1 and 2 and Table 3 will show, the over-all sensitivity in the detection of low-titer anti-D as well as of the rarer antibodies was equal to that of papain and bromelin. Since the technic is based on a well-defined immunological principle,[2, 3, 7] it is mandatory that the serum used contains antibodies not only to gamma but also to alpha and beta globulins since not all hemagglutinins are of the gamma type. The failure of most AHG sera to detect cold agglutinins, which may not be a serious disadvantage in instances other than ABO incompatibility, may be attributed to this. The possibility of elution of antibody as a result of undue delay in adding the AHG serum to the washed cells[1] and the weakening of the reaction by incubation, which is discouraged by American producers of commercial serum, are factors to be considered in interpreting AHG results. The present study indicated that lack of attention to these details of reagent selection and points of technic might be responsible for reports of significantly greater sensitivity on the part of certain enzyme technics. The 30-60

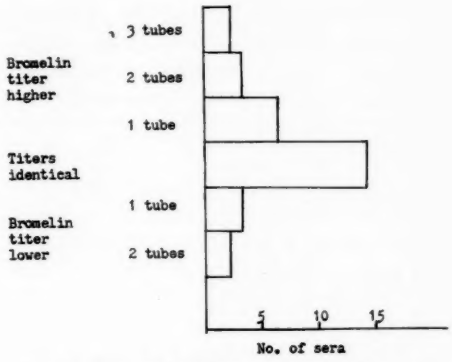

FIG. 3. Comparison of sensitivity of papain and bromelin in titration of 30 anti-D antibodies.

TABLE 5. *Detection of Antibodies in the ABO System*

	Titers							
	Naturally occurring and immune anti-A				Immune anti-A			
Serum	Saline	AHG	Papain	Bromelin	Saline	AHG	Papain	Bromelin
1	96	768	768	768	neg.	12	48	48
2	192	768	1,536	1,536	neg.	24	48	48

Results recorded as the reciprocal of the highest dilution showing a clear-cut "+" reaction.

TABLE 6. *Bromelin and Papain Detection of Antibodies in Random Prenatal Anti-D Sera Absorbed with D-Positive Cells to Remove Antibodies Detectable with AHG Serum*

	Before absorption			After absorption			
Sera	AHG	Papain	Bromelin	AHG	Papain	Bromelin	No. of absorptions
1	256	512	512	0	16	16	2
2	1,000	2,000	2,000	0	32	32	4
3	128	64	64	0	8	16	1
4	256	128	256	0	4	4	2
5	256	1,000	2,000	0	16	8	2
6	128	128	128	0	16	8	3
7	512	128	512	0	4	64	2
8	64	16	32	0	0	8	1
9	64	64	128	0	8	16	1
10	128	32	128	0	0	4	1
11	32	128	128	0	8	8	2
12	256	128	128	0	0	0	1
13	256	256	256	0	0	0	3
14	8	2	8	0	0	0	1
15	256	256	128	0	0	0	2
16	256	256	256	0	0	0	3

Results recorded as the reciprocal of the highest dilution showing a clear-cut "+" reaction.

minute sensitization period followed by repeated washings still seems to be the greatest drawback of the AHG technic. However, French[4] reported that a 15-minute sensitization period detects all clinically significant antibodies.

The papain technics devised by Stern *et al.*[12] seemed to combine specificity, reproducibility, and simplicity with economy of time and material. Accordingly they were used as the starting point for this study, and modifications were made as directed by the results of the experiments. No significant difference in sensitivity was noted between papain activated by cysteine alone and that activated by cysteine and EDTA. Although cysteine in solution undergoes changes that may result in decreased papain activity, particularly at room and refrigerator temperatures, no significant decrease in the activity of activated papain stored at −20 C. for six to eight weeks was noted. Bromelin was found to be more stable than papain both with and without preservatives at both 4 C. and −20 C. as Table 2 shows.

Although duplicate titers using papain and bromelin of the same lot agreed within the limits of experimental error, it was found that different lots prepared by the same method did not always yield identical results. It is recommended, for this reason, that each new lot of enzyme be compared for sensitivity with the one previously used. Only in this way will successive titers be significant.

Lewis[9] noted an inhibition of papain by serum in dilutions up to 1:4 and of bromelin by serum in dilutions up to 1:32. In this study prozones sometimes occurred up to a serum dilution of 1:64 with papain but only up to 1:8 with bromelin. The use of stock papain in dilutions up to 1:32 and dilute papain thereafter seems justified by the enhancement of the titers thereby. The absence of enhancement by the use of dilute bromelin, as well as the basic economy of the 0.5 per cent solution,

would seem to justify the use of stock bromelin throughout.

With high-titered antibodies agglutination was produced by immediate spinning. Prolonging the incubation period beyond 15 minutes decreased rather than increased the sensitivity, indicating thereby that 15 minutes is optimal. Thus the enzyme technics may, at least in some instances, furnish the answer to the request for emergency compatibility testing.

The greater sensitivity of bromelin and papain reported previously as shown by antibody detection several months earlier and by significantly higher titers could not be reproduced with the sera tested. The detection of two anti-E antibodies by papain only was in accord with the findings of Vogt[13] and Heisto[8] but not with statements by Pirofsky[11] and Griffitts[6] on the special effectiveness of bromelin for antibodies of this specificity. On the other hand, the detection of AHG-negative anti-c antibody by both enzyme technics confirmed the findings of these latter workers. Neither papain nor bromelin consistently demonstrated the presence of anti-Kell antibodies even when AHG tests were subsequently performed. This would indicate that at least some Kell antigens and/or anti-Kell antibodies are altered by enzyme activity. Inasmuch as anti-Kell ranks next to anti-D and anti-c antibodies as a cause of transfusion incompatibility and hemolytic disease, this is a noteworthy defect. Pirofsky's consistently positive results with anti-Kidd, anti-Duffy, and anti-S[11] were not duplicated in this study. The detection of some anti-Kells, anti-Duffys, and anti-Kidds by the AHG tests subsequent to enzyme treatment was not considered significant because the AHG test alone detected these antibodies and false positives were likely to result. The decrease noted in anti-M after enzyme treatment was in accord with previous reports of enzyme alteration of the M antigen. In the two cases studied bromelin and papain were superior to the AHG technic in the detection of immune anti-A, thus giving promise of being useful tools in the hitherto difficult diagnosis of ABO incompatibility.

From the experiment in which papain and bromelin-reacting antibodies could be detected after complete absorption of AHG demonstrable antibodies, it appears that at least some of the antibody detected by these technics may be different from the AHG antibody. More work will have to be done, however, before it can be stated with any degree of certainty that differences in specificity as well as sensitivity are operative.

Simplicity in the performance of enzyme technics, economy and stability of reagents used, and marked reduction in the time required were all demonstrated in this study. Adoption of these technics, can for these reasons, be expected to result in extending the benefits of prenatal and pretransfusion studies to a larger number of patients and donors. However, since, as has been stated, antibodies to the Kell and Duffy antigens are not consistently demonstrated thereby, blood banks that are interested in furnishing the best possible transfusion and prenatal service to their patients cannot disregard these inadequacies of the enzyme technics. To discard the AHG technic would result in a gain in time and convenience at the expense of the established advantages of the AHG test. The less striking results obtained in this study as compared with some previously reported investigations might well reflect the use of AHG sera of lower sensitivity and specificity than those commercially available today. Papain and Bromelin Antibody Screening

Conclusions

1. Papain and bromelin technics, although not so specific, sensitive, and reproducible as some reports claim, are satisfactory within the limits applying to any enzyme technic.

2. They are economical of time and material.

3. As a consequence of this economy they are practical for use as screening tests in prenatal and pretransfusion antibody detection.

4. They appear to be logical replacements for the albumin technic.

5. They cannot replace the AHG technic, but they are valuable additions and, in some instances, may make the completion of this test unnecessary.

6. The choice between the bromelin and papain technics depends on individual preferences and circumstances rather than on exactly defined advantages, although bromelin solutions are much more stable at refrigerator temperature.

Acknowledgments

The author is grateful to Dr. J. J. Griffitts for a gift of "Bromelase" and to Dr. R. M. Heinicke of the Honolulu office of the Hawaiian Pineapple Co., Ltd. for a generous supply of "Bromelain."

The material presented is taken from a thesis presented in partial fulfillment of the requirements for the degree of M.S. (Medical Technology) at Ohio State University.

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Book Reviews and Abstracts

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Book Reviews

Electrophoresis in Physiology. Lena A. Lewis. 2nd Ed., No. 385 American Lecture Series. Springfield, Ill., Charles C Thomas, 1960, 120 pp. $5.50.

THE LITERATURE on the subject of electrophoresis has become colossal. Dr. Lewis prepared the first edition of this small book in 1950 to introduce the newcomer to the subject and help him get started. The first chapters discuss the basic principles and major types of electrophoresis which have been developed. Chapter 5 attempts to give a bird's-eye view of the application of electrophoresis to problems in physiology and clinical medicine. The reviewer was left with the impression that the author's desire to cover the material briefly and thoroughly was somewhat blurred by the presentation of non-essential details. The emphasis placed on conditions in which electrophoretic patterns are not specific complicate the picture for the novice. It was surprising to read that "At birth the serum γ-globulin concentration of the baby is higher than that of the mother . . . " On page 57 fibrin is used when fibrinogen is meant. Chapter 6 consists of nine pages on immuno-electrophoresis by M. D. Poulik who is well-known to those interested in this field. It serves the purpose of introducing the subject and stimulating interest. The appendix outlines procedures for performing moving boundary, paper and immuno-electrophoresis and is followed by a list of sources of equipment and supplies. The bibliography is extensive but the author has thoughtfully included a brief listing of general references.

The beginner will find this volume useful as first reading but it is not intended as a reference work.—*T. J. Greenwalt, M.D.*

Recent Advances in Human Genetics. L. S. Penrose. Boston, Mass., Little, Brown and Co., 1961, 194 pp. $8.00.

"THE STUDY of heredity in man, after a long and comparatively quiet period of incubation, has erupted violently during the last decade into a most exciting and productive activity." Thus Dr. Penrose prefaces this absorbing volume, dealing with the latest developments in the study of linkage, chromosome number, and genetic abnormalities of sex, foetal growth, and hemoglobin synthesis. A distinguished group of British and American scientists has contributed to *Recent Developments in Human Genetics,* and their fascinating discussions of the progress being made in the above areas, carefully edited by Dr. Penrose, are both lucid and

comprehensive. Included are descriptions of the laboratory technics used to make chromosomes clearly visible, the newer statistical devices and theories, and the problems of detecting and measuring genes. The bibliography is excellent.—*Hugh Fudenberg, M.D.*

Abstracts

Blood Groups and Antibodies

An Rh Gene Complex, r^M, in Some Ways Like r^G. P. A. Tippett, R. Sanger, I. Dunsford and M. Barber. Lister Institute, London, England. Vox Sang. 6: 21, 1961.

An Rh factor was studied which can be distinguished from other described cells which react with anti-CD sera but not with anti-D and only erratically with anti-C. A large amount of serologic data was collected. The discussion ends with the statement that, with the rapid advance of knowledge of the Rh factors, attempts at explanations of isolated peculiarities such as this are unprofitable.—*P. J. S.*

A Negro Family Revealing Hunter-Henshaw Information and Independence of the Genes for Js and Lewis. R. E. Rosenfield, G. V. Haber, R. Schroeder and R. Ballard. Mount Sinai Hosp., New York, New York. Am. J. Hum. Genet. 12: 2, 1960.

A Negro family revealed the probability of the rare combination of NS, Hu and He on the same chromosome. The father of the family tested as M+ N+ S+ s+ Hu+ He+, the mother as M− N+ S− s+ Hu+ He−. Tests showed that two of four of their offspring received the Hu and He antigens along with the paternal NS, the other two receiving neither with the paternal Ms. This indicates that the father carried NS, Hu and He on one chromosome, Ms on the partnering chromosome. Of the two who received the paternal Ms, one received the maternal Hu, the other did not. The authors discuss the possibility that Hu may be associated with S^u in the mother, but the family gives no evidence in this regard.

The family shows independent segregation of the Js and Lewis antigens.—*M. L.*

Anti-Rh_0(D) Antibodies Produced by Iso-Immunization in an Rh Positive Mother of the Unusual Genotype R^1r^y (CD^ue/CdE). R. T. Simmons and V. Krieger. Commonwealth Serum Laboratories, Melbourne, Australia. Med. J. Australia. 1: 1021, 1960.

The authors add another to the growing list of rare D^u women who produce an anti-D antibody. The antibody which titered 1:2,000 gave apparently typical anti-D reactions although in rare instances it failed to react with known D positive cells. The case is of particular interest since the immunized patient is of the rare Rh genotype CD^ue/CdE.

The patient's first two babies were normal, the third was direct Coombs positive, suffered mild hemolytic disease and required two simple transfusions. The fourth baby was normal, its Rh genotype CdE/cde.—*M. L.*

Serum Protein Types

Le Facteur Serique Gm^a Chez Les Africains de Porto-Novo (Dahomey). A. Eyquem, L. Podliachouk et J. Presles. Institut Pasteur, Paris, France. Vox Sang. 6: 120, 1961.

The authors examined the serum of 116 Negroid Africans in Porto-Novo (Dahomey) for the presence of the serum factor Gm^a. All individuals were found to be of phenotype, Gm (a+).

Quantitative studies revealed that the concentration of Gm^a in the serum of the Dahomenians was less than that found in serums collected from Negroes in Cameroun. The concentration of the substance, Gm, was found not to be directly related to the concentration of gamma globulin.—*P. M.*

Le Facteur Serique Gm^a Chez Les Grecs. L. Podliachouk, G. Angelopoulos et A. Eyquem. Institut Pasteur, Paris, France. Vox Sang. 6: 123, 1961.

A report is made on the frequency of the serum factor, Gm^a, in inhabitants of various parts of Greece. Of 308 individuals studied, 120 (39%) were Gm (a+) and 188 (61%) were Gm (a−). This frequency (39%) is the lowest rate observed so far in the white race although probably not significantly different from the frequency of 40 per cent found in natives of southern Italy. The proportion of Caucasoids of phenotype Gm (a+) is reported to vary from 39 per cent to 67 per cent. The authors point out that the frequency of the Gm^a factor differs according to race. Testing done so far on individuals of the Negroid race from Senegal, Cam-

eroun, Dahomey and America has revealed that nearly 100 per cent are of phenotype, Gm (a+). Likewise the frequency has been found to be extremely high in the Mongoloid race.

The authors indicate that the Gm^a factor in serum is a gamma globulin, demonstrated by its ability to inhibit agglutination of Rh positive red cells (sensitized with incomplete anti-Rh_o) by serum obtained from certain patients with chronic rheumatoid arthritis. If an individual's serum inhibits agglutination of the sensitized cells, his phenotype is Gm (a+); if his serum fails to inhibit agglutination, the phenotypic designation is Gm (a−). Materials and methods for the determination of the Gm^a factor are described.—*P. M.*

An Isoprecipitation Reaction Distinguishing Human Serum-Protein Types A. C. Allison and B. S. Blumberg. Nat. Inst. of Health, Bethesda, Maryland. Lancet. 1: 634, 1961.

THE SERA from 61 patients, who had received multiple transfusions, were tested for precipitins against other human sera. Three samples exhibited typical precipitation lines. Serum precipitins have recently been demonstrated in rabbits immunized with sera from other rabbits.

The methods used included agar gel diffusion and immunoelectrophoresis. Studies on the one serum that gave the strongest reactions is the subject of this report. This serum, from an anemic man who had received approximately 50 transfusions, was found to give strong precipitation with sera of some, but not all normal subjects. An antigen-antibody reaction was believed to be involved. While the patient had had pyrogenic reactions from some of his later transfusions, a correlation between them and the newly discovered precipitin had not yet been looked for.—*J. B. R.*

ERYTHROBLASTOSIS

Experimental Observations on Cardiac Arrhythmia During Exchange Transfusion in Rabbits. W. C. Taylor, C. N. Gillis, C. W. Nash and G. L. Kullman. Univ. Hosp., Edmonton, Alberta, Canada. J. Ped. 58: 470, 1961.

FIFTY-SEVEN adult rabbits were subjected to exchange transfusions. The technic was designed to simulate closely the procedure used in newborn infants. Electrocardiographic tracings were made frequently. Hyperpotassemic citrated blood transfusions resulted in 15 deaths in 19 animals. The usual sequence of events began with prolongation of the Q-T interval, S-T depression and increased amplitude of T waves. Ventricular fibrillation followed and then cardiac arrest. It is believed that this same sequence of events occurs in the newborn human. The early electrocardiographic changes were reversed by the administration of calcium. The administration of citrated blood of normal potassium content or heparinized blood did not result in ventricular fibrillation. The administration of chilled donor blood resulted in recognizable electrocardiographic changes but not in an increased incidence of ventricular fibrillation or a higher mortality.—*J. B. R.*

Research on the Effects of Citral, an Antihistaminic, on the Isoimmunized Organism of the Pregnant Woman. I. A. Vasil'eva. Institute of Obstetrics and Gynecology, Leningrad, Russia. Akusherstvo i Ginekologiya. 35: 24, 1959.

CITRAL, a member of a series of antihistaminics beginning with vitamin A and its provitamin—carotene, was given to 17 isoimmunized women, half of whom were Rh negative. Antibody titers were reduced in all of eleven women in whom therapy was begun early in pregnancy and nine bore healthy infants. In six cases who were treated after the mid-point in their pregnancy, there were five dead children and antibody titers were not reduced.

(The titers in 10 of the 17 patients were 1:2. Reduction in titers did not correlate with clinical effect. The authors report nothing on the specificity of the antibodies and state that in many cases they did not know the blood group and Rh type of the infants. Considering only the Rh negative women, there were five affected and four unaffected children. These women would be expected to have a ratio of Rh positive to Rh negative children of just 5:4 and the experiment is inconclusive.— (Ed).—*P. J. S.*

Hemolytic Disease of the Newborn Due to the Good Factor. B. A. Eskin, E. U. Laufer and M. D. Pettit. Women's Medical College, Philadelphia, Pennsylvania. Am. J. Ob. & Gyn. 81: 997, 1961.

THIS is a further report on the Negro woman in whom anti-Good was first demonstrated (Blood. 15: 681, 1960). Her obstetrical history: By first husband—one and two: normal—By second husband—three: premature, normal—four: premature, died, hemolytic disease—five: abortion, patient transfused—six and seven: stillbirths at seven months, cause unknown.

In the eighth pregnancy, a cesarean section was elected at six and one-half to seven months because of an irregular fetal heart beat; the baby was liveborn but died shortly (cord blood Hb 5.5 Gm. per cent, bilirubin 3.35 mgm. per cent). The direct Coombs was negative. Autopsy confirmed prematurity and hemolytic disease. A routine search for antibodies had been made on the mother's blood and none found: after delivery it was found that her serum agglutinated the husband's and child's cells. The antibody so detected has been tested against 1,700 other blood samples; no tests were positive. The antigen defined differed from all known antigens.

In the ninth pregnancy the patient was given steroid therapy starting at 12 weeks gestation at which time the antibody titer was 1:8. By the seventh month the antibody was not demonstrable. In the thirtieth week cesarean section was elected because of diminution of fetal activity: a 3 lb. 15 oz. girl with a cord blood Hb of 7.4 Gm. per cent and bilirubin of 5.3 mg. per cent was delivered. The direct Coombs test was negative but the baby's cells were agglutinated by the mother's serum. The baby responded well to four replacement transfusions.

The antibody (anti-Good) was inhibited using Low's papain technic. Six further examples of the antibody were found to be naturally occurring.—*M. L.*

Hemolytic Anemia

Immediate and Long-Term Results of Treatment of Chronic Hemolytic Anemias. I. I. Lorie, A. S. Kukel and M. A. Umnova. Central Order of Lenin Institute of Hematology and Blood Transfusion U.S.S.R. Problems of Hematology and Blood Transfusion. 5: 50, 1960.

The authors review their observations on 88 patients with congenital and acquired hemolytic anemias. Thirty-six patients with congenital hemolytic anemia were subjected to splenectomy. Thirty had typical congenital spherocytosis; two died in the postoperative period but each of the remaining 28 had a complete clinical recovery. In these successful cases, hemoglobin reached normal in 25-30 days after operation. In the first 24 hours after operation, eight patients of this group showed a considerable increase in hemoglobin which was attributed to emptying of the splenic reservoir. Bilirubin returned to normal on the fifth day. In spite of clinical recovery spherocytosis persisted. Of the six patients with atypical spherocytosis, four had a recurrence of hemolysis after splenectomy. The authors conclude that splenectomy in congenital spherocytosis should be considered early, while in atypical forms, with normo- or macrocytosis, indications for the operation should be stringent.

Sixteen patients with acquired hemolytic anemia were submitted to splenectomy. Of 11 survivors, eight had favorable results although return to normal was much slower than in congenital hemolytic anemia. Twenty-three patients with acquired hemolytic anemia were treated equally effectively with steroids or ACTH. Eight patients had complete restoration to normal and 11 had partial remissions of hemolysis. Four showed no improvement and were operated upon with three deaths and one remission.

In the authors' opinion early cessation of steroids leads to recurrence. Although Coombs titers declined under steroid treatment in no case did the Coombs test stay negative. Maintenance treatment may be intermittent but must be resumed at the first sign of relapse. All patients with acquired hemolytic anemia should be started on hormones in large and continuous doses; operation should be performed when there is intolerance or refractoriness to steroids. The use of steroids immediately prior to operation makes the results of surgery worse, and the authors consider it desirable to discontinue hormone therapy about 10 days before operation.—*A. K.*

Serum Factors of Acquired Hemolytic Anemia in Leukemia and Lymphoma. J. T. Brody and S. C. Finch. School of Medicine, Yale Univ., New Haven, Connecticut. J. Clin. Invest. 40: 181, 1961.

The purpose of this study was to determine whether the abnormal serum factors of acquired hemolytic anemia in leukemia and lymphoma could be characterized as antibody by the immune adherence technic. Serum samples from a total of seven patients with acquired hemolytic anemia were studied. Direct and indirect Coombs tests were compared with immune adherence antibody assays. The technic of immune adherence, which is positive only when antigen and specific complement-fixing antibody are present, was utilized by reacting erythrocyte antibody or patient's serum with group O red cells in the presence of complement and guinea pig platelets. The platelets, acting as indicator by adhering to the agglutinated red cells, could be observed by dark

field microscopy. Six of the seven patients had positive direct and indirect Coombs reactions, but immune adherence did not occur when the patients' serum was reacted with their own red cells. In addition those serums obtained from untransfused patients did not exhibit immune adherence when reacted with normal erythrocytes. Two patients who had been repeatedly transfused did show such immune adherence. The authors believe that immune adherence is a sensitive indicator for the detection of erythrocyte antibody and that the absence of immune adherence indicated that a specific antigen-antibody reaction was not present in the hemolytic anemia patients studied despite the positive Coombs test. The authors discuss the evidence that abnormal serum factors in acquired hemolytic anemia are not erythrocyte antibodies mentioning the ways in which strict antibody criteria are not fulfilled. It is suggested that the serum factors which have affinity for the erythrocyte surface may represent another manifestation of the dysproteinemic state.—*A. K.*

Transfusion Reactions

Bacterial Contamination of Preserved Blood and Derivatives. P. H. Bonnel. Direction Centrale des Services de Sante des Armees, Paris, France. Vox Sang. 6: 60, 1961.

In 1959, French transfusion centers were surveyed regarding their experiences with transfusion accidents due to bacterial contamination. There were 26 proved cases (with 13 deaths), mostly due to Gram negative bacilli. The products utilized, blood, plasma or albumin, had in general been stored for less than eight days and minimum amounts had been transfused, only a few milliliters in several cases.

Experiences with the variability of the bactericidal effects of blood, the effect of bacterial toxins, and with cold growing bacteria were reported. As regards culture technics, there was a note on the necessity for testing for anaerobic bacteria and fungi. In one series of cultures, in large part of used containers, 33 per cent of the contaminants were fungi.

The following resolutions were adopted by the French Society of Blood Transfusion: (1) Each accident should be investigated and reported; (2) blood containers should never be entered before transfusion; (3) cell suspensions should be made with rigorous asepsis; (4) blood derivatives must be subjected to sterility tests; and (5) strict personnel discipline must be applied at all stages in the production of blood derivatives.—*P. J. S.*

Severe Haemolytic Transfusion Reactions Caused by Apparently Compatible Red Cells. F. Kissmeyer-Nielsen, K. B. Jensen and J. Ersbak. Arhus Kommunehospital, Arhus, Denmark. Brit. J. Haemat. 7: 6, 1961.

A patient with several severe hemolytic transfusion reactions is reported. Despite apparent compatibility of the transfused cells by all available technics, Cr^{51} survival studies revealed immediate elimination of the compatible donor cells, whereas the patient's own cells were eliminated at a normal rate. The literature dealing with similar instances is also reviewed.—*H. F.*

Experimental Transfusion of Donor Plasma Containing Blood-Group Antibodies into Incompatible Normal Human Recipients. I. Absence of Destruction of Red-Cell Mass with Anti-Rh, Anti-Kell and Anti-M. II. Induction of Iso-Immune Haemolytic Anaemia by a Transfusion of Plasma Containing Exceptional Anti-CD Antibodies. J. F. Mohn and R. M. Lambert. School of Medicine, Univ. of Buffalo, Buffalo, New York; H. S. Bowman and F. W. Brason, Harrisburg Hospital, Harrisburg, Pennsylvania. Brit. J. Haemat. 7: 112 and 130, 1961.

The purpose of this study was to gather experimental data to determine whether the irregular antibodies that would be picked up by screening all donors for antibodies in preference to doing a minor crossmatch are a sufficient hazard to the recipient to warrant the time involved in such a screening.

Plasma from donors selected for the presence of specific antibodies was stored frozen if not for early use. The recipients, male student volunteers, were selected on the basis of desired blood groups, non-sensitization, normality of cell survival and hematological and clinical normality. A donor antibody to be given was titrated against the cells of the recipient selected to receive it.

Pre-transfusion studies were made of cell survival and red cell mass. To determine post-transfusion effects of the antibody donation, estimations were made of cell survival, red cell mass, plasma Hb, serum bilirubin, reticulocyte per cent and detection of sensitization of red cells and of free circulating antibody.

A control series of seven antibody-free plasmas, prepared in the same way as were the antibody-containing plasmas, was run to determine the effect of such transfusions on the survival curve. Of the experimental series seven

plasmas contained Rh antibodies, four anti-D, two anti-DC and one anti-c; two plasmas contained anti-K and one anti-M. In each case sensitization of the recipient's red cells did occur, but no circulating antibody was detected. No unequivocal evidence of red-cell lysis (see methods above) was found nor were any clinical symptoms of reaction apparent. The authors conclude that the magnitude of reaction in these recipients would not necessitate preliminary screening of donor sera as a routine blood bank procedure. They further conclude that while these were the results in normal recipients there is no reason to believe that these results would be different in ill patients, provided no intrinsic red-cell defect existed.

The subject of the second paper, an anti-CD antibody plasma, was exceptional in two respects: the donor was a woman of Rh genotype $cD^{u}e/cde$; the titer was one of the highest ever encountered by the authors. The antibody gave its highest titers by the serum + albumin method: vs. Cde/cde ($R'r$) 1:512; vs. cDe/cde ($R_{o}r$) approx. 1:1 million; vs. $C^{w}De/.$ CDe ($R_{1}^{w}R_{1}$), the recipient, approx. 1:0.5 million.

The recipient was selected and studied in the same way as had been the previous members of this study. However, in contrast to the others, he developed signs of acute red cell destruction: the mean cell life was 4.9 days; up to day seven there was an estimated total loss of 1,680 ml. of red cells. Pallor and mild jaundice developed, but splenomegaly was not detected. Urobilinogen increased in the urine. Spherocytes were observed in the smear. The peak of reticulocytosis on the fifteenth day was 26 per cent; the peak plasma Hb was on the first day—24.5 mgm. per cent, the peak serum bilirubin on the third day—3.2 mgm. per cent.

The recipient's cells gave a strong serum + albumin (Witebsky) test and direct antiglobulin test immediately after transfusion. These gradually diminished in strength, the first disappearing on day 105 and the second on day 140. Free antibody as detected by the indirect antiglobulin test against the recipient's cells and against $R'r$ and $R_{o}r$ cells reached its peak on day five, but was no longer detectable by day 14. On day 91 an antibody demonstrable by this method appeared and persisted till some time between days 140 and 198, but this antibody reacted only with the recipient's cells by this method during this period. In contrast the antibody demonstrable by the serum + albumin (Witebsky) technic was present from day one to day 140 but had disappeared by day 198. Its titer, however, had three peaks centered around days one, 25 and 120, and it reacted with all three test cells *in vitro*. Curiously the third rise began just at the time when the direct test by this method for *in vivo* sensitization became negative, yet, though the titer of the antibody using this method reached 512, evidence of *in vivo* sensitization did not again appear. The patient developed no symptoms at the time of the final appearance or rise of antibody.

The authors discuss at some length the meaning of the third rise in titer, questioning whether it could represent specific active autoimmunization or an antibody augmentation phenomenon.

The authors conclude that *exceptional* Rh antibodies in donor blood, although having minimal hemolytic activity, may sequester and destroy circulating red cells of a normal recipient.—*M. L.*

Coagulation

The Problem of Fibrinolysis. L. D. Orlova, Central Order of Lenin Institute of Hematology and Blood Transfusion, U.S.S.R. Problems of Hematology and Blood Transfusion. 5: 593, 1960.

After a brief review of the fibrinolytic mechanism and its importance in certain types of abnormal bleeding, the authors note that, during a study of leukemia, delayed bleeding resembling fibrinolytic hemorrhage was observed. Fifty patients with various forms of leukemia were studied, and, in six, fibrinolysis was confirmed by a clot lysis test. Fibrinolysis was also detected in one patient with hemorrhagic thrombocythemia and in one case of lupus erythematosis. Of the six patients with leukemia, two had chronic myeloid leukemia and four had acute blastic leukemia. Coagulation studies of the eight patients with fibrinolytic activity showed that fibrinogen was decreased below 100 mg. per cent in three and prothrombin ranged below 70 per cent in five patients. Reasons for the development of fibrinolysis in leukemia are discussed, the authors favoring the theory that gross changes in the liver disturb the production of anti-fibrinolysin with consequent activation of fibrinolysis. Treatment of fibrinolysis in leukemia by vigorous therapy of the underlying disease with steroids, 6-MP, myleran and x-ray is advocated. When remission is achieved, fibrinolysis ceases. Direct action against the fibrinolytic process by the use of fresh whole blood and plasma is also advocated. The authors conclude that fibrinolysis

as well as thrombocytopenia may be a cause of serious hemorrhage in leukemia.—*A. K.*

MISCELLANEOUS

Polyolefin Plastics As Blood Filters. H. H. LeVeen, B. Schatman and F. Falk. Vet. Admin. Hosp., Brooklyn, New York. Surgery. 49: 510, 1961.

THIS BRIEF report describes an investigation of platelet loss that occurs during passage of whole blood through commercially available administration set filters. A new filter of woven polyethylene fibers was designed and compared with the latter. Counts showed platelet losses of approximately 40 per cent after single passages of 300 ml. of blood through each commercial filter, virtually none through the polyethylene filter. Moreover, since no blocking of the interstices occurs with the polyethylene filter, this type could be used for many units of blood. Thus, for those patients who should receive a maximum number of platelets, a polyethylene filter is to be preferred.—*J. B. R.*

Benzalkonium Chloride As a Source of Infection. W. F. Malizia, E. J. Gangarosa and A. F. Goley. Walter Reed Army Hospital, Washington, D. C. New Eng. J. Med. 263: 800, 1960.

A GRAM negative bacillus, incriminated in infections in eleven patients, was recovered from a container of 1:750 aqueous solution of an antiseptic (Zephiran®) in which gauze squares were kept. It was also found in three separate one liter bottles of the solution. The gauze was used to cleanse the skin before venipunctures and, in three of the patients, the organism was recovered in pure culture from abscesses as well as from the blood stream.

An experiment was performed which proved again that the presence of gauze reduced the bactericidal effect of the aqueous benzalkonium chloride. The tincture was not so affected. The practice of soaking gauze sponges in the solution is a real health hazard.—*P. J. S.*

***Letters to the Editor:* Nickel Sensitivity.** P. V. Marcussen. Lancet. 1: 670, 1961.

THE AUTHOR supports Stoddart's recent observation of sensitivity to nickel in cannulae as a cause of allergic transfusion reactions. Among 621 patients with nickel sensitivity, the cause in 14 was considered to be injection needles. In four of these the relation was striking. Eleven other cases were found in medical personnel who handled surgical instruments.—*J. B. R.*

Electronic Demonstration of Blood Cell Agglutinins. M. J. Halloran, W. J. Harrington, V. Minnich and G. K. Arimura. Washington U. Sch. of Med., St. Louis, Missouri. Amer. J. Clin. Path. 35: 105, 1961.

AGGLUTININS for red cells, platelets and white cells can readily and reproducibly be demonstrated with an electronic counter (Coulter Counter, Model A), now available in many hematology laboratories, by appropriate scaler settings which exclude recognition of unagglutinated cells. The method does not distinguish between non-immunologic aggregation of cells and agglutination due to antibodies; in this respect a major shortcoming of agglutination reactions in general remains unresolved.—*H. F.*

Questions and Answers

R_z and R_y

Q. "How are R_z and R_y bloods identified?" —*M. T., Detroit, Mich.*

A. "Only those red cell specimens which are agglutinated by both anti-C and anti-E antibodies can be R_z or R_y positive; this is characteristic of about 12 per cent of specimens donated in most North American blood banks. A very small number of these (considerably less than 1 per cent) may be genotyped immediately by their serological reactions:

C+D−E+c+e−	*CdE/cdE* genotype
C+D−E+c−e+	*CdE/Cde* genotype
C+D−E+c−e−	*CdE/CdE* genotype

Another small number can be concluded to be either R_z or R_y positive or both:

C+D+E+c+e−	*CDE/cDE, CDE/cdE* or *CdE/cDE*
C+D+E+c−e−	*CDE/CDE* or *CDE/CdE*
C+D+E+c−e+	*CDE/CDe, CDE/Cde* or *CdE/CDe*

This leaves only two additional patterns of serological activity:

C+D+E+c+e+	*CDe/cDE, CDe/cdE, cDE/Cde, CDE/cde, CDE/cDe* or *CdE/cDe*
C+D−E+c+e+	*CdE/cde* or *Cde/cdE*

The D negative phenotype is very uncommon, representing the alternative genotypes *CdE/cde* or *Cde/cdE*. However, those cells which react with all five antisera are found in nearly 12 per cent of the population, and constitute the major problem in genotype differentiation. On the basis of statistical probability, by far the most likely genotype of such cells is *CDe/cDE*, followed by *CDe/cdE* and *cDE/Cde*. The remaining possible genotypes are either R_z or R_y positive:

CDE/cde, CDE/cDe or *CdE/cDe.*

All are very rare. Certain additional antisera may be used to distinguish this rare trio from their commoner alternatives. For example, "anti-f" usually recognizes the presence of *c* and *e* on the same chromosome. Therefore, if cells in this serological category are agglutinated by "anti-f," it may be concluded that they are probably R_z or R_y positive (*i.e.* the homologous chromosome carries both *C* and *E*). Furthermore, certain antibodies with apparent anti-C specificity will only agglutinate cells from individuals having *C* and *e* (but not *C* and *E*) on the same chromosome. This antibody has been called anti-rh_1 or anti-Cē; in most instances it is a reliable guide in differentiating *CDE/cde, CDE/cDe* and *CdE/cDe* (negative reaction) from *CDe/cDE, CDe/cdE* and *cDE/Cde* (positive reaction). This would be equally true of *CdE/cde* (negative) and *Cde/cdE* (positive).

Such presumptive findings should be supported, whenever possible, by family studies. These are frequently, but not invariably, helpful in working out Rh gene segregation.

Note: The above discussion does not take into account such possibilities as gene deletion, suppression or mutation, nor does it intend to imply that the individual letter symbols, used for convenience, necessarily represent any well defined segments of chromosomal DNA.—Eloise R. Giblett, M.D.

Warming Blood

Q. "I would appreciate a discussion on the matter of whether bank blood for replacement transfusion in hemolytic disease of the newborn should be given cold, immediately from the blood bank refrigerator, or whether the blood may be warmed to near body temperature before administration."—*M.D., Portland, Oregon.*

A. "Death from hypothermia is a very real danger during exchange transfusions of the newborn, and prior warming of stored bank blood is essential for the success of the procedure. The method of warming that appears to be the most satisfactory, is to pass the donor blood through coils of tubing that are immersed in a water bath heated to 38-40 C. If the content of blood immersed is equal to the volume of each cycle of the discontinuous exchange, the reservoir of donor blood will remain cold while the volume of blood reaching the infant with each cycle will be at body temperature. The water bath must be thermostatically controlled, the water should be circulated by pump or convection, and the operator must be certain that the bath has not been filled with water that was too hot. Waterbaths of this description are manufactured or can be made, and recipient sets with 16-20 feet of tubing are available (as is sterile disposable plastic tubing of proper length)."—*R. E. Rosenfield, M.D.*

Crossmatch in Auto-Immune Hemolytic Anemia

Q. "Comments on crossmatching blood for patients with auto-antibody, patients with acquired hemolytic anemia and others where the direct and where also the indirect Coombs test is positive will be appreciated."—*M. T., Montreal.*

A. "Before considering the problems of crossmatching in patients with idiopathic acquired hemolytic anemia, it seems appropriate to recall the recommendations of Dr. Scott Swisher: to withhold blood transfusion in these cases until it is clearly essential to prevent immediate death, to give steroids, to use bed rest and oxygen, if necessary to enable the patient to get along with the low hemoglobin concentration, and to use small transfusions rather than massive ones, when transfusion has become absolutely necessary. In my own experience, massive transfusion has stimulated marked 'autoantibody' formation in some cases, and should be avoided if possible. A patient may survive without transfusion, even with a hemoglobin as low as 2 grams, if supportive treatment is good and he is in good general health. Massive transfusion may raise the hemoglobin concentration for a long enough period to allow bone marrow hyperactivity to subside, thus possibly delaying recovery.

It is important, in these patients, to identify any specific antibody they may have. Anti-e is the most commonly encountered specific antibody in these cases, and can be identified by absorbing the serum (or eluate from patient's

cells) with e-negative red cells, to remove 'non-specific' antibody, then testing against several known e-negative and several e-positive bloods. Other antibodies may be identified by similar procedures. By selecting especially useful bloods for the absorptions (a blood that is O, e-negative S-negative, P-negative, Fy^a-negative, Jk^a-negative would be especially useful, for example), it may be possible to rule out a number of antibodies at one time. This is time-consuming work, and requires access to bloods of useful types. If specific antibodies are found, donors lacking the corresponding antigens should be selected for crossmatch.

In performing the cross-match itself, it is advisable to match several bloods, if possible, and to test each one with serial dilutions of the patient's serum, selecting for use that donor whose red cells give the lowest 'titer' with the patient's serum."—*Fred H. Allen, Jr., M.D.*

Typing of Recipient

Q. "Regarding the D^u factor of recipients of a blood transfusion would you advise screening all patients who are Rh_o (D) negative for the D^u factor. If D^u positive would you then advise to genotyping the patient to show the so-called 'gene interaction' type or 'hereditary' type in order to decide the type of blood to be transfused. Would you advise Rh_o positive blood with c-negative to recipients of D-negative, D^u-positive, c-negative?"—*M. T., Montreal.*

A. "I have for a long time advised against routine testing for D^u in *recipients* because few D^u's are missed by standard Rh typing tests with commercial anti-D serums now available and because of the relatively high risk of false positives in such testing. If one were dealing with a population in which D^u is a frequent type, and *recipients* were to be tested for D^u in such populations, control tests should include the direct Coombs test on each person, and tests to insure, so far as is possible, that the typing serum contains no antibody but anti-D. So far as I know, there is no need to distinguish the inherited D variants from D^u's caused by gene interaction. I would advise against trying to supply c-negative blood to c-negative recipients in this, as in all other 'routine,' situations." —*Fred H. Allen, Jr., M.D.*

Problems

Q. "1. How are properly identifiable sterile pilot tubes attached to plastic bags prior to bleeding?

2. Are there any blood banks that inspect every bottle every day for visual evidence of contamination or hemolysis and how do they keep the records?

3. What is considered to be a satisfactory method for indicating that ABO groupings and Rh typings have been repeated on pilot tube samples received from outside agencies?

4. Would you describe in detail the methods used for sterility tests?

5. What precautions are necessary to avoid falsely identifying a recipient as Rh positive?" —*M. D., Minnesota.*

A. "1. The attachment of properly identified pilot tubes to plastic bags prior to bleeding depends upon the type of bag. A bag with integral donor tubing obviously has this length of donor tubing as an attached pilot tube. In addition, any plastic bag can have a pilot tube attached before bleeding utilizing adhesive plaster, cellulose tape, or any other device which securely attaches the pilot tube to the bag. In a long experience with one type of plastic bag which also has integral donor tubing, several blood banks with a large volume have had no difficulty in carrying out the prior attachment of a pilot tube.

2. 'The Standards for a Blood Transfusion Service of the AABB' states 'Each container of blood shall be visually inspected at *regular* intervals during storage and immediately prior to release for undue hemolysis, abnormal cloudiness or color.' The significant inspection that is required for the 'Standards' is that which is given immediately prior to release. A regular but not necessarily a daily overall inspection is required, but this does not have to be a searching examination of each container. A simple record indicating that a daily inspection of the refrigerator and its contents have been made is adequate. The transfusion release form may be used to indicate the final satisfactory appearance of the blood.

3. A satisfactory method to indicate that the ABO group and Rh type has been repeated on bloods received from outside sources is to leave the original grouping label attached and visible, and to apply a tie-tag with the grouping performed by the final agency.

4. A suitable method for sterility testing is the following:

(a) Blood intended for transfusion should not be tested for sterility by a method that requires entrance of the final container before the blood is used for transfusion. If such a method is used, the blood in the entered container must

be used within three to 24 hours in accordance with the 'Standards for a Blood Transfusion Service.'

(b) The sterility test itself should be performed regularly at least once monthly and should include containers of blood that appear normal. The latter should be sampled within the 18th to 24th day after collection. The volume of the sample should be adequate, preferably 5-10 ml., inoculated into a total volume of fluid thioglycollate or thioglycollate broth medium 10 times the volume of the sample of blood. It should be incubated for seven to nine days at a temperature of 30-32 C. and subcultured on the third, fourth, or fifth day utilizing thioglycollate for seven to nine days at a temperature of 30-32 C.

(c) Instead of performing one test using an incubation temperature of 30-32 C., two tests may be performed; one at an incubation temperature at 18-22 C., and one at an incubation temperature of 35-37 C.

Walter and his associates have described a simple method for sterility testing of plastic bags with an integral donor tube. In this method a 1 ml. sample is harvested from the integral donor tubing, incubated for 10 days, and then streaked on blood agar and observed 24 hours later. This method may be found in the New Eng. J. Med. 257: 364, 1957.

5. To avoid falsely identifying a recipient as Rh positive, one must employ a control system utilizing the recipient's cells in its own serum and handling this system in the same manner as the test with the anti-Rh serum. The use of saline-active anti-Rh serum will also avoid false positives due to auto-agglutinins, hyperglobulinemia and other causes."—*Mark Falcon Lesses, M.D.*

Sale or Service

Q. "Would you be kind enough to forward me a sample transfusion authorization that implies over-all hospital service rather than sale?" —*M.D., Texas.*

A. "There are many differing authorization forms for blood transfusions. However, I believe that the one prepared by the Law Department of the American Medical Association and published on page 16 of its book entitled 'Medical Legal Forms—With Legal Analysis' is as complete and legally sound as such a form can be."—*Howard Hassard, San Francisco.*

Meeting Notes

Blood Groups in Infrahuman Species

A CONFERENCE on Blood Groups in Infrahuman Species was held on May 15 and 16, 1961 in New York City under the auspices of the New York Academy of Sciences, Section of Biological and Medical Sciences. This conference, well organized by Dr. Carl Cohen of the Batelle Memorial Institute of Columbus, Ohio, and Dr. D. B. Amos, Roswell Park Memorial Institute, Buffalo, New York, provided one of the first opportunities for workers dealing with blood groups of infrahuman species in the course of laboratory investigation on one hand and in relation to agricultural animals on the other to exchange views and delineate a number of basic common problems. On the whole, the conference enjoyed a stimulating and forward-looking viewpoint and an informal atmosphere which evoked substantial discussion.

Several major themes emerged from the presented papers and accompanying discussion. The first of these was blood grouping and its possible relationships to histocompatibility in a variety of experiments involving tissue transplantation in mammals. Although a great deal of work has been done on several aspects of this question, much more needs to be known about these relationships, particularly in higher mammals where inbred strains are not available, before blood grouping can be used as a major guide to experimental designs. A second group of papers dealt primarily with immunogenetic problems. It is apparent that the full force of blood grouping as a genetic tool will be realized first in animals where controlled breeding studies permit much more precise description of the genetic mechanisms involved in the transmission of heritable blood group antigens. An especially interesting aspect of the latter are the efforts to correlate blood groups with a variety of economically important characteristics of domesticated animals. Serological stud-

ies of blood group relationships between closely related species in an effort to understand a variety of evolutionary and ecological relationships emerged as a third rather tenuous line of study being undertaken presently. Finally, a number of papers which were primarily descriptive of a wide variety of blood grouping systems encountered in many different species of animals were presented. Many of these latter studies provide the necessary preliminary work before other genetic, immunological or experimental investigations are undertaken in a wide variety of new species. It was from these latter studies that the listener was impressed with the tremendous amount of work presently in progress in the blood groups of infrahuman species and the fundamental and broad biological importance of blood grouping in general. Blood groups seem to appear wherever they are looked for.

The first day's program was devoted primarily to blood group studies of laboratory animals. Space does not permit detailed review of even a majority of the papers presented. Rhesus and a variety of other primates, rabbits, rats, mice, and dogs were the principal laboratory species discussed. Most of the antigens that have been identified have been utilized primarily as markers in a variety of transplantation, transfusion, or genetic experiments. Cohen has approached the problem of the relationship of antigenicity of a variety individual of red cell antigens to the total antigenic constellation of the animal's erythrocytes utilizing the rabbit. This work appears to have great importance in understanding the wide variability in antigenic potential of the many red cell antigens of all species, including man. A number of practical problems encountered in the dog and in rabbits related to experimental transfusions or production of viable offspring free of hemolytic disease of the newborn, were also discussed. Virtually all of the investigations reported during the entire conference involved the use of iso-antibodies produced by immunizing animals within a single species; in a few instances heteroantibodies with specificity for blood grouping antigens have been employed. "Naturally occurring" antibodies of the sort encountered in the human ABO system apparently are very uncommon in other species, a fact which may well be related to the much more recent development of blood grouping studies in these species.

Papers presented by Drs. George Ridgeway and William Hildemann demonstrated the feasibility of blood grouping studies even among the fishes, amphibia and reptiles. The extraordinary technical and practical difficulties facing these workers gave cause for gratitude to many investigators dealing with more tractable species.

The second day's program was devoted to blood grouping studies of animals of economic importance. Four papers attested to the great current interest in the blood groups of chickens. Interesting correlations between blood groups and economically important characteristics such as egg laying and weight gain are under investigation. Briles presented a group of studies utilizing an approach to the problem of specificity of reagents which is novel in the field of human blood group studies. By development of a large panel of reagents all reacting to one or more antigens within a single complex system, differential patterns of reactions can be observed and multiple antigens within the system can be recognized. Gilmour presented data on several genetic studies, including linkages and factors involved in polymorphism, using seven blood groups in the species. Studies of red cell and histocompatibility antigens, availability of lectins which distinguish blood groups in chickens and the demonstration of polymorphisms of serum and egg albumin proteins all suggest that the chicken may be potentially a more important laboratory animal than is commonly recognized for studies involving these problems. Scheinberg presented a very interesting and important paper dealing with the "HI" agglutinogen in chickens. This agglutinogen appears in female chickens after the onset of egg laying and its activity seems to depend upon the estrogenic level in the animal. Injections of diethylstilbesterol given to young birds can result in premature transformation of their red cells to an agglutinable status. Moreover, males possessing this genetic potential can also have their red cells transformed to an agglutinable form by stilbesterol injections. This work underscores again the wide variety of possible mechanisms whereby a serologically detectable erythrocyte "antigen" can be controlled by non-genetic mechanisms.

Blood grouping studies of cattle were dealt with by Drs. Clyde Stormont and William Stone. The wide variety of blood grouping antigens occurring in this species is well known to most students of blood groups. Over 350 antigens, belonging to a relatively small number of independent systems, have been recognized in cattle. Stormont noted that at the present time it is theoretically possible to recognize over two

trillion phenotypes in cattle. The staggering complexities of blood groups in this species almost fade the complexity of human blood grouping systems into insignificance. Further studies of the very intriguing J system of cattle were presented by Dr. Stone. The J substance is a soluble blood group substance probably a glycoprotein, which becomes absorbed to the red cells from the serum. This system again points out the diversity of the mechanisms by which red cell antigens are produced. J positive cattle can now be divided into two groups, based upon a high or low content of J substance in the serum. The factors which control the absorption of the J substance by the erythrocytes appears to reside in the serum of the animal and not in its red cells.

Blood grouping studies of pigs, horses and sheep were also reported on the second day of the program. The occurrence of hemolytic disease of newborns in pigs and horses has stimulated much of the work in this field. The blood groups of sheep at present involve seven separate systems and appear also to be highly complex. An interesting homologous relationship appears to exist between the B system of sheep and the B system of cattle. In both species the system is extremely complex, involving at least 52 phenogroups in sheep.

The conference was closed by a paper presented by Dr. Andre Eyquem and colleagues surveying their work of the last 15 years on blood groups of a variety of animal species. The important contributions made by these French workers are especially well known among workers in the field of human blood grouping.

A problem also very familiar to workers in the field of human blood grouping rose repeatedly during this conference, that of nomenclature systems. Following a dinner in honor of Professor M. R. Irwin, whose own work and whose students have contributed so much to this field, a meeting was held devoted to an informal discussion of the problems of blood grouping nomenclature. This session, presided over by Dr. Amos, heard brief reviews of the major problems of nomenclature from workers dealing with the several species. It promptly became apparent that a wide variety of philosophies of nomenclature were represented. In the main, however, the simplest possible notations have been employed at the outset of work on various species. As the blood grouping systems developed in complexity, many of the nomenclature devices have become increasingly inadequate. Thus, the letters of the alphabet have been used to designate blood groups in virtually every species!

It appears that blood grouping workers have been strongly motivated to employ notations closely allied to those of the pure geneticist. Whereas this has certain advantages in establishing a common language with the discipline of genetics, it has other disadvantages, particularly in that it may imply a great deal more genetic knowledge of the situation than is actually known. It is increasingly difficult, particularly in species such as cattle and sheep, to specify in detail the antigenic content of the red cells of any individual. Thus, certain shorthand notations are developing by convention within these areas. Almost immediately it became apparent that a great deal of thought must be given to designing notation systems in the future and that many of the present notation systems must be revised. Most urgently there seems to be need of agreement on a set of principles for devising notations which will be applicable to all species. All of the investigators present appeared to have very great interest in this problem and indicated willingness to cooperate in any way necessary to reach a satisfactory resolution. To this end, it was recommended that a small working group be established within the newly founded society devoted to immunogenetics to study this problem in detail and to recommend methods of formulating these principles. If such an agreement can be reached in the field of infrahuman blood grouping, and if the system can be demonstrated to be workable, a major contribution may be made to the field of human blood grouping where the problems seem to be qualitatively no different.—*Scott N. Swisher, M.D.*

1961 Meeting of the American Society of Human Genetics

THE ANNUAL meeting of the American Society of Human Genetics was held in Atlantic City at the Claridge Hotel, May 3-5, 1961. The afternoon session on Thursday, May 4th, was held jointly with the Society for Pediatric Research, emphasizing the increasing importance of human genetics for pediatrics and clinical medicine.

The papers of interest to the audience of this Journal included those dealing with blood

groups, chromosomal abnormalities and plasma protein polymorphisms. P. H. Saldanha used blood groups to ascertain the Negro, Portuguese and Indian admixture in northeastern Brazilian populations. J. A. Buckwalter presented secretor studies on the ABO blood group disease association. K. Mayeda was unable to obtain any correlation between the mother's Rh type and the ability of the daughter to produce Rh antibodies which suggests that immunological tolerance does not play a role in maternal-fetal Rh incompatibility.

N. Wald and collaborators presented an epidemiologic and cytogenetic study of the relationship between Mongolism and leukemia. They confirmed an increased incidence of leukemia in Mongolism in all age groups throughout life. P. Mamumes reported a case of acute myelogenous leukemia in a 38-year-old male with Klinefelter's syndrome, suggesting that an increased susceptibility to leukemia may be due to an excess of sex chromosomes in addition to an excess or imbalance of autosomes (Mongolism).

A. G. Steinberg presented evidence that indicates anti-Gm^a reagin obtained from healthy donors detects the same factor as reagin obtained from rheumatoid arthritics even though the two reagins differ both qualitatively and quantitatively. H. Cleve and A. G. Bearn indicated that the mode of inheritance of group specific alpha-2 component is compatible with a two allelic system without dominance. Elegant biochemical studies were applied by E. Sutton and B. Bowman to two genetic types of transferrin in an attempt to find a difference in their primary protein structure.

Other papers of interest included a report by R. Hall and associates in which about one-third of the parents and sibs of patients with antibody positive thyroid disease had demonstrable thyroid autoantibodies even though they were free of thyroid disease. J. A. Wolff and W. A. Bauman presented biochemical studies on a patient with acanthocytosis (thorny erythrocytes) which indicated that this disorder represents a deficiency or absence of beta-lipoprotein and that the red cell abnormality is secondary to the defect in lipid metabolism.

One of the highlights of the meeting was the presidential address by Professor L. C. Dunn entitled "Crosscurrents in the History of Human Genetics," which deserves to be published.—*S. P. Masouredis.*

News and Announcements

Preliminary Program for the 14th Annual Meeting of the American Association of Blood Banks

Administrative Section

The first two sessions will be panel discussions with questions from the floor answered by the panelists.

SESSION I—Thursday, October 26, 1961, 9:30 a.m. to 12:30 p.m.

A. Blood Service Plans Including Blood Procurement Programs
For Discussion: Functions, services and benefits by the people from diverse geographical areas who participate in all phases of blood banking.

B. Hospital and Community Blood Banks
For Discussion: Their functions and the role they have in the community. Are there too many blood banks? Benefits of a community blood bank or vice versa.

SESSION II—Friday, October 27, 1961, 2:00 p.m. to 5:00 p.m.

A. Blood Bank Administrative Problems (Question and Answer Workshop)
A few of the selected questions to be discussed:

1. How can local blood banks secure greater support in publicizing their work (public relations rather than public information)? 2. Exploitation of donors and recipients. 3. Types of records and their importance. 4. Blood donor replacement policies. 5. Donor procurement for massive transfusion, extracorporeal surgery, etc., and 6. Blood inventory (excessive outdating and continuing to have an adequate inventory for normal needs to meet emergencies).

SESSION III—Saturday, October 28, 1961, 9:00 a.m. to 12:00 noon.

A. Paper Presentations
1. National Clearinghouse Agreement. 2. Rare Donor File (correct procedures, etc.). 3. Requirements for Certification in Blood Banking and Establishment of a School for Specialty Certification. 4. Report from Inspection and Accreditation Committee—Progress and Problems. 5. "A Word to the Wise," and 6. Recent Regulations Issued by the Department of Health, Education and Welfare.

Scientific Program

Guest speakers from abroad will be Drs. Fred Stratton, John F. Loutit and R. R. A. Coombs who are well known to our readers.

Time schedule not shown because it is not finalized. Most presentations will be limited to 15 minutes. Discussion periods and intermissions have been omitted to conserve space.

THURSDAY, OCTOBER 26, 1961

MORNING SESSION

Israel Davidsohn, M.D., *Chairman*

Blood Groups—Antibodies

A Simple and Practical Method for Concentrating Blood Group Antibodies, *Eloise R. Giblett,* Lucy E. Brooks*

The Effectiveness of Technics in Demonstrating Isohemagglutinins, *James J. Griffitts,* Polly Schmidt*

Enzyme Effects on the Agglutinability of Red Cells Sensitized with Incomplete Antibody, *S. P. Masouredis*

The Function of Proteolytic Enzymes and Tannic Acid in Inducing Erythrocyte Agglutination, *Maria Cordova,* Theodore L. Imel, Bernard Pirofsky*

Studies of Specificity of Human Isoantibodies, *Neva M. Abelson,* Arnold J. Rawson*

Effects of Magnetic Fields on Erythrocyte Agglutination, *Emanuel Hackel,* Adolph E. Smith*

Serological Characteristics of Rh-Positive Cells Determined with Rh Antibody Fractions Separated by Deae-Cellulose Column Chromatography, *Mitsuo Yokoyama, Joel M. Solomon,* Eleanor Morrison*

Detection of the r′ (Ce) Chromosome in a Rh_o (D)-Positive Family by the Quantitative Hemagglutination Method, *Mary B. Gibbs*

Transplantation

The Transfer of Lymphoid Cells, *John F. Loutit*

AFTERNOON SESSION

Kurt Stern, M.D., *Chairman*

Transfusion Practice

A New Method for Preparing Buffy Coat-Poor Blood, *Tibor J. Greenwalt, Mieczyslaw Gajewski, James L. McKenna**

Experience with Newly Designed Equipment for Preparation of Buffy Coat-Poor Red Cell Suspensions for Transfusion, *Mona Cassell,* Dorothy R. Phillips, Hugh Chaplin, Jr.*

Preliminary Screening of Blood Donors with the Microhematocrit Test for Red Cell Volume and the Plasmacrit Test for Syphilis (HCT-PCT Method), *Rita L. Don, Don W. Orlowski, John B. Alsever**

The Occasional Fallibility of *in Vitro* Compatibility Tests, *Hugh Chaplin, Jr.,* Mona Cassell*

Preliminary Observations on Possible Toxic Effects of Citrate Ion, *E. R. Jennings, et al.**

Limits to Plasmapheresis as a Form of Blood Donation, *Allan Kliman,* Lawrence A. Gaydos, Emil J. Freireich*

Recent Developments in Laboratory Studies of Human Hepatitis, *I. Wm. McLean, Jr.,* Alton R. Taylor, Wilton A. Rightsel*

Serologic Studies in Burned Human Subjects ("Toxin-Antitoxin"), *Sol Roy Rosenthal,* Wilma A. Spurrier, George T. Crouse*

Denaturation of Protein by *in Vacuo* Drawing of Blood, *Dieter Krumhaar, William H. Lee, Jr.,* James V. Maloney, Jr.*

Film

Blood Fractions in Clinical Medicine, *Produced by Synergist Productions, Inc.*

Discussants: *Edward L. Burns, John J. Clemmer, Coye C. Mason*

FRIDAY, OCTOBER 27, 1961

MORNING SESSION

Tibor J. Greenwalt, M.D., *Chairman*

Blood Groups—Anthropology

Distribution of Hereditary Blood Factors Among Indians of Mexico. II. Tzotzil and Other Maya, *G. Albin Matson,* Jane Swanson*

Blood Groups—Genetics

A Survey of the D^u Problem in a Heterogeneous Population, *Phillip Sturgeon*

Identification of Chromosomes by Blood Grouping Tests, *Fred H. Allen, Jr.*

Gene Frequencies of Blood Group Antigens in an Expanding Population, *David E. Soules*

Blood Groups—Antibodies

The Detection, Activity and Nature of Blood Group Antibodies, Which Fix Complement, *Fred Stratton*

Blood Groups—Genetics

Report on a Family with Six Tj^a-Negative Siblings, *Kurt Stern,* Shirley Busch*

Current Status of the Vel Blood Group System, *Leon N. Sussman*

The Relation of Human and Cattle Blood Groups, *William H. Stone*

AFTERNOON SESSION

E. R. Jennings, M.D., *Chairman*

Blood Groups—Immunology

Immunization to Rh_o (D) Observed in Pregnancy of a Woman with Type rh^G (G), *Douglas W. Huestis,* Kurt Stern*

An Uncommon Antigen and Common Antibody, Bg, *Bruce Chown,* Marion Lewis*

* To present paper.

Bone Marrow and Hemopoiesis

Erythropoietin, *Leon O. Jacobson,* Clifford W. Gurney*

Transplantation of Marrow and Whole Organs, *E. Donnall Thomas,* David A. Blumenstock, Joseph W. Ferrebee*

Autologous and Isologous Bone Marrow Infusion in Man as an Adjunct to Tumor Therapy, *N. B. Kurnick*

Blood Groups—Immunology

Normal Survival of Incompatible Red Cells in the Presence of Anti-Lu^a, *Robert M. Greendyke,* Frank W. Chorpenning*

The Rate of Sensitization of Erythrocytes *in Vitro*, *Anthony V. Pisciotta, et al.**

Kinetics of the Reaction between Human Serum and a Highly Purified Anti-Human Gamma Globulin Reagent, *J. M. Hill,* David E. Soules, Dan Hampton*

The Non-Immunologic Reaction of Globulin Molecules with the Erythrocyte Surface, *Bernard Pirofsky,* Theodore L. Imel, Maria Cordova*

The Competition Theory of Tolerance, *John Gorman,* James Chandler*

SATURDAY, OCTOBER 28, 1961

MORNING SESSION

William C. Levin, M.D., *Chairman*

Blood Groups—Disease

Correlation between Rh Blood Groups and Ulcerative Colitis, *William C. Boyd,* Mary Heisler*

Alteration of Erythrocyte Antigen B in Acute Leukemia, *Allyn B. Ley,* Jean P. Harris*

A Review of Clinical and Investigative Experience with Cold Agglutinins in Disease, *William C. Levin,* Stephan E. Ritzmann*

Rh-D Hemolytic Disease in Negro and White Infants—A Difference, *Lyndall Molthan*

Data Pertaining to the Presence and Significance of Fetal Erythrocytes in Maternal Circulation, *Flossie Cohen,* Wolf W. Zuelzer*

Hyperbilirubinemia Due to Exchange Transfusion in the Newborn, *Albert Ehrlich*

James F. Mohn, M.D., *Chairman*

10:50-11:35 (Title to be announced), *R. R. A. Coombs*

The Effects of Blood *p*H on the Transport of Bilirubin, *Arthur Sawitsky,* Eli Seifter, Stefan Stein*

Blood Groups—Genetics

Further Studies on the Hereditary Gamma Globulin (Gm) Groups in Man, *H. Hugh Fudenberg*

* To present paper.

AFTERNOON SESSION

John B. Ross, M.D., *Chairman*

Proposal for a Neutral Blood Group Terminology: A Panel Discussion, Philip Levine, M.D., *Chairman*

A New Rh Terminology, *Richard E. Rosenfield*

New Notation for the Kell System, *Fred H. Allen, Jr.*

2:40-250 (Title to Follow), *Scott N. Swisher*

Discussion to Be Opened by: *Alexander Wiener, Fred Stratton*

General Discussion

Leukocytes

The Relation of Newborn Leukocyte Counts to Maternal Leukoagglutinins, *Rose Payne*

Application of *Limulus* Serum to Simple, Rapid Preparation of Leukocyte Suspensions for Immunological Study, *Elias Cohen,* Maria A. Galley*

Fever, Pyrogenicity and Leukotoxicity in Transfusion Practice, *Paul J. Schmidt,* Sherwin V. Kevy, Mary H. McGinniss*

Israel Davidsohn, M.D., *Chairman*

Preservation of Blood and Blood Fractions

Comparison of the ACD and Heparin Systems for Blood Storage, *Charles Bishop*

A Comparison of the Antigenic Integrity of Fresh Red Blood Cells, Glycerolated Red Blood Cells, and Red Blood Cells Stored in Liquid Nitrogen, *William R. Bronson*

A Comparative Study of Factors Influencing the Stability of Factor VIII (Antihemophilic Globulin) in Bank Blood and Frozen Plasma, *Herbert A. Perkins,* D. J. Acra, Mary R. Rolfs*

Technical Section Program

(Time schedule not final and intermissions not shown)

Wednesday, October 25

Preconvention Seminar

Basic Blood Group Serology

Julius Rutzky, M.D., *Chairman*

Fundamental Genetic Principles: Chromosomes, Genes, Alleles, Gene Frequency, Calculations, *Emanuel Hackel, Ph.D.*

Blood Group Genetics: Gene Frequency, Calculations, Linkage, *Emanuel Hackel, Ph.D.*

A-B-O-H System: A B O H, Isoantibodies, Types, Secretion and Neutralization (exclusive of Lewis), *Polly Schmidt, M.T. (ASCP)*

A-B-O Subgroups: A_1, A_2, etc., Inhibitory Genes, Reagents, Lectins Absorbed B Serum, etc., *Grace Neitzer, M.T. (ASCP)BB*

Rh System: Rh CDE, Inheritance, Antibodies, Homozygous *vs.* Heterozygous, *Patricia Corcoran*

Rh Subgroups: Types C^w, D^u, etc., *Corinne Hindmarsh, M.T. (ASCP)BB*

Lewis System: Relation to ABO, Serum, Saliva, Cells, Inheritance, Newborn, Antibodies, *Shirley Busch, MPH, BB Sp. (ASCP)*

Panel Discussion

Thursday, October 26 through
Saturday, October 28

Blood Bank Bazaar

9:00-5:00 A special feature of the Tection Section Program will be the "Blood Bank Bazaar." Various booths, manned by members proficient in the practical field of blood banking, will be set up so that participants can perfect technics and solve problems in ABO and Rh typing. A special bazaar attraction will be the "Answer Man," in which outstanding authorities in the field of immunohematology will answer questions from the attending members.

The booths will be open throughout each of the three days, thus permitting registrants to attend the bazaar at their convenience. This will be a real opportunity to obtain expert help with problems of technic.

Friday, October 28

Technical Paper Presentation

From Donor to Patient: Another Safety Factor, *Barbara Butterfield, LCDR, MSC, USN*

Reducible Minimum, *DeWitt T. Hunter, Jr., M.D., Kathryn T. Holland, BB (ASCP)*

Serum Haptoglobin: A Preliminary Study, *E. Taylor Peterson, A.B., Max Kroger, B.S.*

The Testing of Donor Bloods for Erythrocyte Antibodies, *Helen Nelson, M.T. (ASCP)*

Trends in Blood Utilization: *John W. King, M.D., Ph.D., Donald A. Senhauser, M.D.*

Clinical and Laboratory Response to Platelet Infusions, *Paul G. Weil, M.D., A. G. Kendall, M.D., R. Baldry, B.S.C.*

Non-neutralizable Anti-A and Anti-B in Human Blood Donors, *James J. Griffitts, M.D.*

Additional Program Notes

Wednesday, October 25, 7-8 p.m. There will be a no-host cocktail hour and "mixer."

Thursday, October 26, 8 p.m. Annual Membership Business Meeting—only members may attend.

Friday, October 28, 6:30-7:30 p.m. No-host cocktail hour. 7:30—Annual Banquet and ENTERTAINMENT! This will be another "fun night" (like last year). Details are not ready for announcement, but it definitely will be on the "light side!"

1961 Blood Banking Certificants

Eighteen technologist members of the AABB, having successfully passed the recent examination in blood banking, have been granted certification by the American Association of Blood Banks and the American Society of Clinical Pathologists. They are as follows:

Mrs. Mary Ellen Brinkley, BB (ASCP) Jamaica, New York

Miss Ruth Marie Gorman, M.T. (ASCP) BB, Lakewood, Ohio

Mrs. Roberta G. Harrison, BB (ASCP), Harvard, Massachusetts

David M. Childers, M.T. (ASCP) BB, Scottsdale, Arizona

Miss Sally Frank, M.T. (ASCP) BB, Miami, Florida

Frank C. Gardiner, BB (ASCP), Connersville, Indiana

Mrs. A. E. Lance Hill, M.T. (ASCP) BB, San Francisco, California

Miss Grace E. Hill, M.T. (ASCP) BB, Roseville, Michigan

Miss Frances W. Holladay, M.T. (ASCP) BB, Richmond, Virginia

Miss Norma Jean Lyons, M.T. (ASCP) BB, St. Petersburg, Florida

LCDR Edna E. McCormick, M.T. (ASCP) BB, Queens Village, New York

Miss Janet M. Summerlin, M.T. (ASCP), BB, Greensboro, North Carolina

Mrs. Betty Van West, M.T. (ASCP) BB, Salt Lake City, Utah

Miss Dorothy M. Pfender, M.T. (ASCP) BB, Philadelphia, Pennsylvania

Peter Rosendale, M.T. (ASCP) BB, Tacoma, Washington

Miss Bernice L. Smith, BB (ASCP), Sacramento, California

Miss Arden M. Stanbury, M.T. (ASCP) BB, Livonia, Michigan

Miss Vita Ann Zodin, M.T. (ASCP) BB, Albuquerque, New Mexico

The total group of newly certified blood bank technologists numbered 38 and to each of them, the AABB extends congratulations.